THE ISOLATION AND IDENTIFICATION OF FILAMENTOUS FUNGI FROM DISTRIBUTION SYSTEMS, GROUND WATER, CISTERNS AND HEMODIALYSIS WATER

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ABSTRACT

THE ISOLATION AND IDENTIFICATION OF FILAMENTOUS FUNGI FROM DISTRIBUTION SYSTEMS, GROUND WATER, CISTERNS AND HEMODIALYSIS WATER

Tammy J. Liles, M.S.
MOREHEAD STATE UNIVERSITY, 1987

Filamentous fungi incidence in 121 water samples from distribution systems, ground waters, cisterns, and a hemodialysis water purification system were assessed over a five-month period. Physiochemical and bacteriological parameters were also examined.

Filamentous fungal colonies were isolated and enumerated by membrane filtration technique using Rose Bengal Agar and CZApek-Dox Agar, isolation media. Of the 121 samples collected, 66% were positive for filamentous fungi. The majority (67%) of positive samples contained more than one fungal isolate. The mean colony forming unit (CFU) for chlorinated and unchlorinated samples were 17.25 and 148.2 respectively. The most frequent genera isolated were Acremonium (18.5%), Cladosporium (16.7%) and Aspergillus (19.3%) in chlorinated, whereas in unchlorinated waters Acremonium (14.8%), Trichoderma (13.4%), Fusarium (8.3%) and

Cladosporium (8.3%) were the most frequently isolated.

Two distribution systems were compared. Source water (creek) for a university system had an average count of 335 CFU's per 100ml compared to 42.7 CFU/100ml for municipal raw water (river). However, finished water (clearwell) yielded an average of 3.8 and 0.25 CFU/100ml for university and municipal system, respectively, a significant decrease from source water. The municipal treatment process using chemical coagulation and disinfection was more efficient than rapid sand filtration. However, distribution water in the university system demonstrated a 1.3 fold increase with the municipal system exhibiting a 5.0 fold increase. Forty-five percent of the university system samples were positive for filamentous fungi while 65% of the municipal system samples demonstrated fungal growth. Acremonium, Aspergillus and Cladosporium were the most frequently isolated genera in the two distribution systems.

Ground water samples (dug wells, drilled wells, and springs) were 88% positive for filamentous fungi ($\bar{x}$ = 51 CFU/100ml). All dug wells and spring samples were positive, averaging 78 CFU and 56 CFU/100ml, respectively, the average CFU for drilled well samples was only 3.3. Acremonium and Trichoderma were the

most frequently isolated while Phoma and Phialophora were the most numerous.

A hemodialysis treatment system was examined by comparing pre-purification (chlorinated) and purified (reverse osmosis) water samples. Pre-purified water exhibited little fungal (0.5 CFU/100ml) and bacterial growth. However, samples collected after reverse osmosis indicated a significant increase in filamentous fungal (35.5 CFU/100ml), yeast (283.3 CFU/100ml) and bacteria (432,700 CFU/100ml) populations. Filamentous fungi most prevalent were Acremonium (49.6%) and Exophiala (37.1%). Rhodotorula was isolated as well as Pseudomonas. Samples collected following disinfection of the dialysis system resulted in a decrease in filamentous fungal (15.5), yeast (11.5) and bacterial (359,000) populations.

An isolation media comparison revealed that RBA demonstrated higher recovery values (57%) than CZApek-Dox agar (43%). RBA demonstrated a shorter incubation period (2-3 days) at 25°C, whereas noticeable growth was not observed until the fourth and fifth days of incubation on CZApek-dox agar. RBA recovered all genera isolated except Helicomyces; whereas, CZApek-dox did not recover Absidia, Alternaria, Dreschlera, Pithomyces, Sporothrix and Trichophyton.

The total data analysis using single and multivariate regression found fungal population to be significantly correlated with heterotrophic plate counts and chlorine levels. However, no correlation was found between fungi present and total coliforms recovered. Therefore, HPC as well as fungal populations, may be a more suitable microbiological indicator of water quality.

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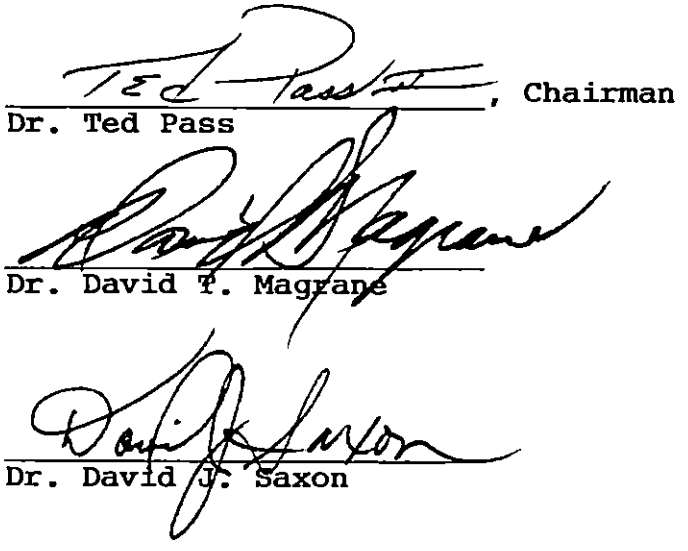

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CHAPTER I

INTRODUCTION

Significance

Federal drinking water regulations recognize total coliform counts as the only microbiological health-associated criterion for potable water quality (American Public Health Association, 1985). Yet in 1984, 26 water-borne disease outbreaks involving 1,755 individuals were reported to the Center for Disease Control, Atlanta, Georgia (CDC, 1985). Of the 26 outbreaks, 35% were of unknown etiology. Total coliform counts may no longer be reliable drinking water quality indicators.

Recently, attention has been drawn to fungi as a potential contaminant of drinking water supplies and their possible harmful effects on health and water quality. However, the significance of fungi in water systems is largely unknown. Fungi are present in and have been recovered from diverse, remote and extreme aquatic environments. Because fungi are enzymatically equipped to utilize a wide range of complex substrates that inadvertently enter drinking water distribution lines, fungi have immense implications for human health.

Fungi are important potential infection agents in man. Eighteen species of fungi are known to cause systemic

and potential fatal mycoses, while severe localized cutaneous or lymphatic infections can result from twelve known species. Superficial infections of skin associated with keratinized tissues can be caused from twenty fungal species (Kishimoto and Barker, 1969). Many common environmental fungi, such as Aspergillus fumigatus and Cladosporium trichoides, are etiological agents of primary mycoses in man (Beneke and Rogers, 1980; Emmons, 1979). Some saprophytic fungi (opportunistic fungi), although generally harmless to healthy individuals, may cause secondary infections in patients under prolonged antibiotic or hormone therapy, immunosuppressed or immunocompromised persons (e.g., Acquired Immune Deficiency Syndrome). or elderly individuals.

Need for the Study

Due to the limited information available regarding the role of fungi in distribution systems, unchlorinated waters, and water utilized by health facilities, there is a need to learn more about fungal incidence following water treatment processes. Also, fungal survival and colonization in water distribution lines, as well as their significance in untreated water needs to be investigated. A review of the literature (Chapter II) indicated that efforts to isolate fungi from distribution

systems or from unchlorinated potable water (wells, springs, and cisterns) in Kentucky have not been reported. Therefore, it is important to ascertain the degree of fungi present in different water sources. Also, it is important to identify specific fungi and their possible effect on human health.

Objectives of the Study

Objectives of this study incompassed various aspects of water quality. They include:

1. To document the presence of opportunistic fungi in waters in health-related institutions (hospital, dialysis clinic, and life care center) where the host could be immunosuppressed and more susceptible to fungal infection.

2. To compare fungal parameters between two distribution systems utilizing different water sources (Triplett Creek/Licking River) and filtration methodology (sand/anthracite).

3. To evaluate the quality of drinking water in selected eastern Kentucky sites by surveying wells (drilled/ dug), cisterns and springs.

4. To compare the presence and types of filamentous fungi in untreated and treated water sources.

5. To determine the efficacy of the water treatment process in the reduction of fungi from source water.

6. To contribute much needed data to the literature and establish the need for further investigations regarding the role of the fungi in potable water.

7. To assess bacteriological water quality utilizing total coliform and heterotrophic plate count techniques.

8. To determine if a correlation exists between bacteriological and fungal parameters.

9. To compare Rose Bengal Agar (RBA) and CZApek-dox agar with respect to genera isolated and numbers of colony forming units (CFU's).

10. To heighten awareness of water quality problems in eastern Kentucky.

CHAPTER II

LITERATURE REVIEW

Occurrence of Fungi in Distribution Systems

There are only five papers surveying fungi in distribution systems. Bays, Burman and Lewis (1970) studied a Great Britain water system utilizing chlorinated surface water and found fungi present in service mains. Cephlasporium (Acremonium), Verticillium, Trichoderma, Nectaria, Phoma, and Phialphora were the most common isolates (See Table 1). This study, however, was restricted to resolving taste and odor problems.

Nagy and Olson (1982) conducted a study using a chlorinated surface water system and an unchlorinated ground water system in Orange County, California. Using the membrane filter (MF) technique, they isolated 24 genera of filamentous fungi on CZApek-dox agar. The mean number of fungal colony forming units (CFU's) was 18 in the unchlorinated system and 34 in the chlorinated system per 100ml of filtered drinking water. Acremonium, Paecilomyces, Penicillium, and Sporocybe (See Table 1) were found to be the most commonly isolated genera. There was no correlation between the presence of fungi and various physiochemical and bacteriological parameters in the unchlorinated system. In the chlorinated system,

Table 1. Fungi Commonly Isolated in Surveys of Potable Water*

Bays et al. (1970)	Nagy/Olson (1982)	Neimi et al. (1982)	Rosenzweig (1986)	West (1986)
Cephalosporium	**Acremonium**	**Aspergillus**	**Alternaria**	**Alternaria**
Nectaria	**Paecilomyces**		**Aspergillus**	**Aspergillus**
Phialophora	**Penicillium**		**Cladosporium**	**Cladopsorium**
Phoma	**Sporocybe**		**Epicoccum**	**Exophiala**
Trichoderma			**Penicillium**	**Phoma**
Verticllium			**Verticillium**	

*Media: Bays - Martin's Acid Rose Bengal Agar
Nagy - Czapek-dox Agar
Rosenzweig - Rose Bengal Agar
Neimi - Malt Extract + Rose Bengal Agar
West - Sabouraud-dextrose Agar

a correlation between fungal frequency, turbidity, and pH was observed. They suggested that this correlation may be due to the effects of one outlier turbidity-pH-fungal frequency value observed at one sampling site.

Neimi, Knuth, and Lundstrom (1982) studied surface (lakes and rivers) water samples and potable water in Finland. Fungi isolated from raw water sources included low concentrations of thermophilic strains (primarily Aspergillus fumigatus). Sand filtration and disinfection using sodium hypochlorite were shown inadequate to remove the fungi (fungi remained present in 29 of 32 treated water samples and 30 of 32 tap water samples). However, chemical (coagulation and disinfection) treatments were more effective in removing fungi from raw water. Fungi were present in 4 of 12 treated water samples and 7 of 12 tap water samples; therefore, disinfection did not prevent fungi from reaching the distribution system.

Rosenzweig, Minnigh, and Pipes (1986) investigated five chlorinated groundwater systems in Pennsylvania and New Jersey. Both filamentous fungi and yeast were isolated using the MF technique and Sabouraud dextrose -rose bengal-streptomycin agar. They found that 49.8% of the 207 collected samples were positive for fungi, with a typical count range between 1 and 6 CFU per 50ml water sample. Filamentous fungi accounted for 96.6%

of the isolates (13 genera) and yeast 3.4% (3 genera). The most commonly isolated genera included Alternaria, Aspergillus, Cladosporium, Epicoccum, Penicillium and Verticillium (See Table 1). The study revealed no correlation between fungi isolated and various physiochemical and bacteriological parameters.

West (1986) investigated a distribution system in the Southern Nevada Water System. Both yeast and filamentous fungi were isolated using the MF technique and Sabouraud dextrose agar. She found 17.6% of 978 collected samples containing filamentous fungi. Only yeast were isolated from the remaining 29% of positive samples. The most common filamentous fungal genera isolated included Cladosporium (27%), Phoma (19%), and Alternaria (7%). The majority belonged to the class Deuteromycetes and the average CFU was 1.5/100ml sample. West found no correlation between positive and negative samples for chlorine, temperature, heterotrophic plate count, pH, and turbidity.

It is apparent from the limited studies conducted that fungi are indeed present in raw water and can survive the stressed environment of water treatment operations. The majority of isolated and identified fungi belong to the class Deuteromycetes and are among those commonly isolated from soil. The spores of these fungi are

ubiquitous and their occurrence in water systems is an indication of their euryhydric ability to survive.

Survival of Fungi

Fungi can enter water distribution systems in treated water or through standpipes and elevated storage tanks. Distribution system storage facilities normally provide an air-water interface, permitting the exchange of air between the tank and atmosphere. Because soil fungi spores are ubiquitous and airborne, they should be found in water distribution systems. Methods for completely eliminating fungi from distribution systems have yet to be discovered.

There is very little information on which to base any evaluation of fungal survival in potable water distribution systems. Rosenzweig, Minnigh, and Pipes (1986) data (See Table 2) show fungal occurrence in various parts of water systems. The composite was developed using samples from five different water systems and may not represent any single system. The storage tanks and clearwells were all open to the atmosphere and fungi were found in a very high percentage of the samples. In addition, the average fungal count per positive sample was also highest in storage tanks. The frequency of occurrence of fungi in samples from residential taps

Table 2. Fungal Isolation From Different Locations Within Water Systems

Part of System	# Samples	% Positive	Average CFU per Positive Sample
Raw Water	25	60.0	11.0
Aerated Water	3	66.7	1.5
Finished Water	7	28.6	2.5
Clearwell	4	75.0	1.7
Residential Tap	152	44.1	1.8
Fire Hydrants	11	81.8	5.9
Storage Tanks	5	100.0	20.0
Total - All Samples	207	49.8	5.5

Source: Rosenzweig et al. (1986)

was much lower, perhaps indicating some die-off in the distribution system. Another study by Rosenzweig, Minnigh, and Pipes (1983) found that fungal conidia and yeast cells react with chlorine in the water. Their data indicated that chlorination in the range of 1.0 to 2.0mg/l was sufficient to eliminate most fungi present in raw water samples. Most fungal conidia and vegetative yeast cells are inactivated under ideal laboratory conditions by chlorine levels ranging from 1.0 to 3.0mg/l. However, their study found some conidia and yeast cells survived when chlorine levels exceeded 3.0mg/l. Thus, the chlorine needed to reduce fungal count by 99.9% would depend on fungal titer in the water.

Significance of Fungi in Distribution Systems

Fungi in water systems might have one of the following effects:

A. Reduction of chlorine levels: the presence of significant amounts of fungal growth could react with chlorine, thereby reducing the chlorine demand of fungal conidia to a range of 3.6×10^{-9} to 3.2×10^{-8} mg/condium while vegetative yeast cells have a chlorine demand of 1.2 to 8.0×10^{-9} mg/cell (Rosenzweig, Minnigh, and Pipes, 1983).

B. Production of chlorinated organics: the reaction

of chlorine with fungal spores, mycelia, or metabolites released into the water could produce chlorinated organic compounds. Some of the compounds produced by such reactions (trihalomethanes) might be a health concern (Rosenzweig, Minnigh, and Pipes, 1986).

C. Interaction with coliforms: some coliform bacteria may be able to grow on the surface of fungal mycelia and could be protected from chlorine by extracellular organic matter produced by the fungi. Slime that accumulated on the inside of redwood storage tanks was found to contain Klebsiella pneumoniae and Enterobacter sp. embedded in fungal mycelia (Seidier, Morrow, and Bogley, 1977). Coliform bacteria surviving on fungal mycelia could give a false indication of sewage contamination of drinking water.

D. Deterioration of joint material: some fungi may be able to colonize and decompose the gaskets used in joints between pipes in a distribution system. Burman (1965) demonstrated fungi isolated from a distribution system could grow underwater using mastic jointing compounds used in concrete expansion joints as the substrate. A Fusarium sp. and the human pathogen, Petriellidium boydii, were found growing on polyurethane caulking used to seal leaking joints in a water main in southern California (Bays,

Burman, and Lewis, 1970). Addition to the pipeline of an initial dose of 350-400 ppm of chlorine and 3 days contact time failed to eliminate the pathogen.

E. Taste and odor problems: it has been suggested by Bays (1965) and by Bays, Burman, and Lewis (1970) that fungi (Acremonium, Verticillium, Trichoderma, Nectaria, Phoma, and Phialaphora) may be partially responsible for various taste and odor problems occurring in distribution systems. This is especially true if the piping is locally warmed and subjected to stagnation or low flow rates for long periods of time.

Human Disease and Mycotoxin Production

While water treatment plants and distribution systems seem to be a less than ideal habitat for the survival of human fungal pathogens, fungi can enter water distribution lines during construction, passing through the treatment processes (Nagy and Olson, 1982) by means of leaks, via sand filtration and from air in contact with water in reservoirs (Hutchinson and Ridgeway, 1977). Evidence suggests that microhabitats (e.g., pits or cracks on the inner surface of the pipe, tubercles, or suspended silt and detritus particles) can serve as sites for growth and colonization by microorganisms (Ridgeway and Olson,

1981; Kennedy, 1971; Tuovinene et al., 1980). In some cases the organisms were attached via the production of extracellular slime. These sites could also serve as loci for fungal growth and the slime material could be used as a substrate for the production of toxic fungal metabolites. In addition, tubercle material in corroded iron pipes has been found to contain humic substances and have an organic carbon content of approximately 2% (Cooke and Kabler, 1953).

It has also been demonstrated that when point-of use water treatment filters (commonly sold for attachment to home water lines, faucets, or various recreational vehicles and boats) are used, they tend to trap and concentrate organic material present in the water. These filters become ideal areas for colonization by microorganisms. The microbial count of the filter effluent increased, especially if the water was not used for a period of time [(e.g., overnight) Tobin, Smith and Lindsay, 1981]. The potential exists for fungi to colonize these filters and pose a health hazard.

Fungi, because of their omnipresence and versatility, are important agents of infection in man [(Bergen and Wagner-Merner, 1977); See Table 3]. Of interest to note among the fungi isolated from distribution systems by Rosenzweig, Minnigh, and Pipes (1986) were eight strains

Table 3. Opportunistic Pathogenic Fungi and Associated Diseases

Genera	Disease
Alternaria	Asthma
Aspergillus fumigatus	Aspergillosis
Aspergillus niger	Otomycosis, Keratomycosis
Aspergillus sp.	Aspergillosis
Cephalosporium sp.	Keratomycosis
Cladosporium sp.	Keratomycosis
Fusarium	Keratomycosis
Geotrichium candidium	Geotrichosis
Phialophora sp.	Chromablastomycosis
Rhodotorula glutinis	Endocarditis

Source: Bergen and Wagner-Merner (1977)

of Aspergillus flavus, two strains of the human pathogen Aspergillus fumigatus and eleven isolates of Aspergillus niger, also a pathogen. The isolates of Aspergillus flavus were all tested and found to be strong aflatoxin (carcinogen) producers.

Fungi have been implicated in allergic reaction epidemics. Water from a steam producing system in a sauna contained the fungus Pullaria, shown to cause an epidemic of allergic alveolitis (Atterholm et al., 1972). Symptoms resembling extrinsic allergic alveolitis caused by potable water was reported in Sweden (Metzer, Patterson, and Roberts, 1976) and in Finland (Muittari et al., 1980). Fungi could not be ruled out as the etiological agent in these epidemics.

Roesch and Leong (1983) isolated fungi on polyurethane caulking used to seal leaking joints of a water main in southern California. One of the fungal isolates was the human pathogen Petriellidium boydii. Certain evidence has supported the belief that the distribution of Histoplasma capsulatum, the etiological agent of histoplasmosis, is related to water sources (Ridgeway and Olson, 1981). Opportunistic and pathogenic fungi have been isolated from ocean areas and a fungus infected with a Herpes-type virus has been isolated from an estuarine environment (Kazama and Schornutein, 1970).

The pathogenicity of fungi to humans becomes more pronounced after prolonged drug therapy or debilitating diseases, such as carcinomas, tuberculosis, or injuries to subcutaneous tissue and skin (Bergen and Wagner-Merner, 1977). Since pathogens and toxin producers have been found in potable water, the use of this water in the preparation of foods, bathing and showering, in mist humidifiers, and for drinking purposes could lead to the introduction of the pathogen into the body (e.g., mouth, ear, lungs). This can be of greater importance when the water is being used by the young, old, or compromised host.

Numerous opportunistic infections have been associated with AIDS. Fungal infections most commonly associated with AIDS includes candidosis and cryptococcosis (Gong and Rudnick, 1986). Both Candida spp. and Cryptococcus spp. were isolated from a southern Nevada distribution system by West (1986). Rosenzweig et al. (1986) demonstrated that Cryptococcus laurenti was also present in water. Holmes and Noble (1986) reported that an AIDS patient in Kentucky had disseminated histoplasmosis as well as infections due to Candida albicans and Cryptococcus neoformans. Aspergillosis has been observed in AIDS patients with Aspergillus niger and Aspergillus fumigatus as the etiological agent (Pervez et al., 1985).

CHAPTER III

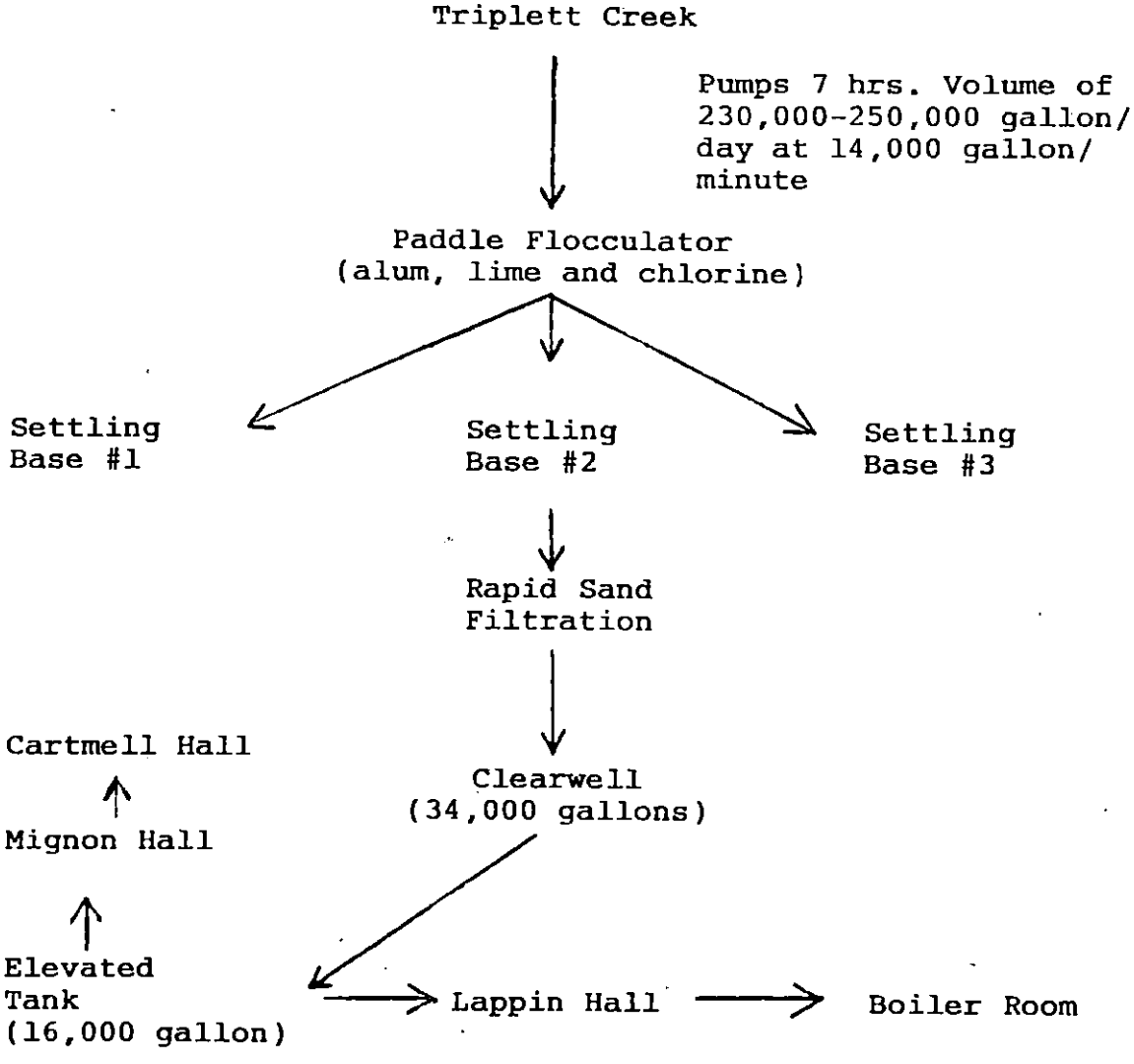
MATERIALS AND METHODS

Collection Sites

Water samples used in this study were collected between October, 1986 and February, 1987 from the following sources:

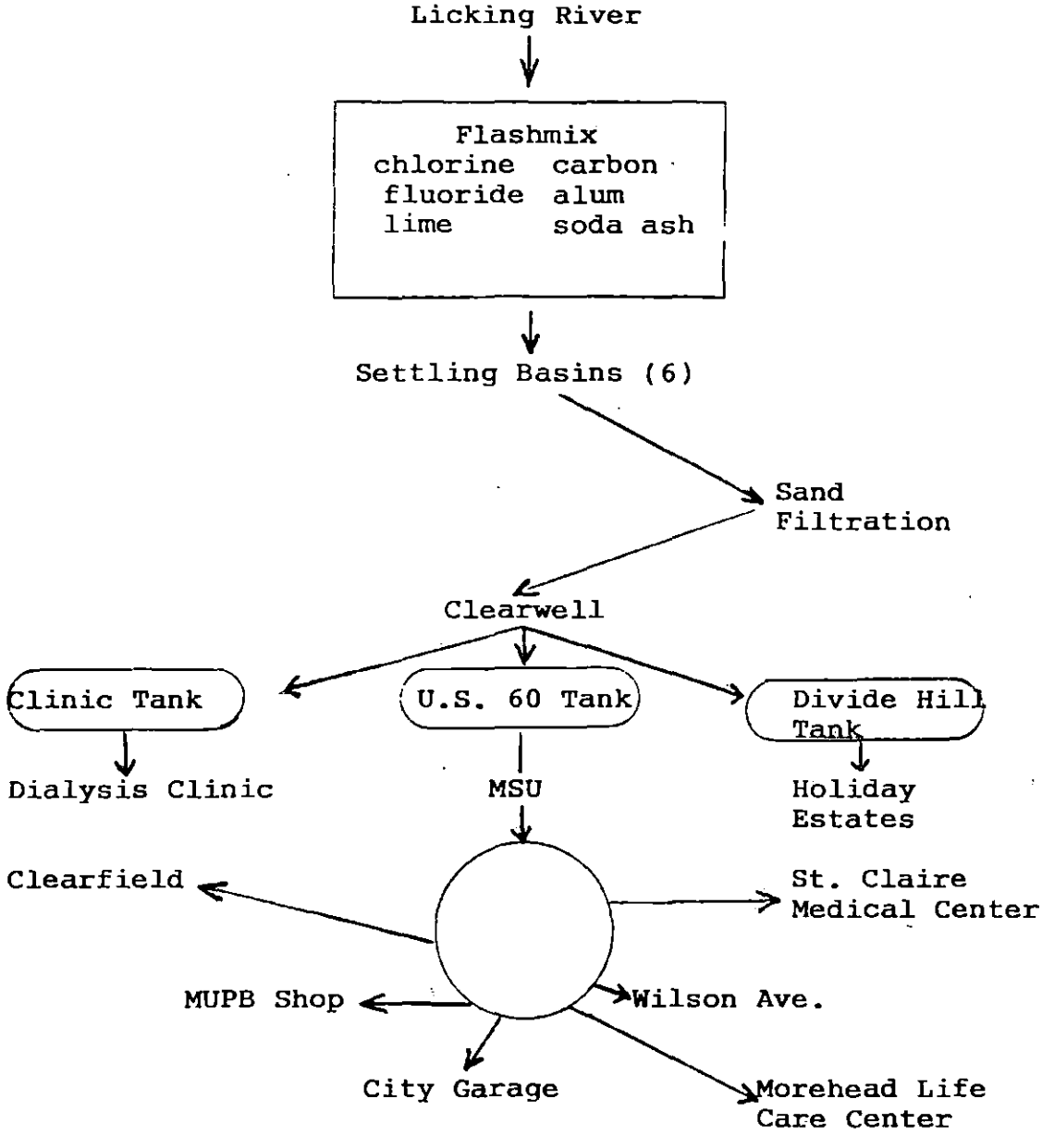
1. Morehead State University Water System (See Diagram 1), collected from:
   a. raw (Triplett Creek)
   b. clearwell
   c. storage tank
   d. points of distribution: Cartmell Hall, Mignon Tower, Lappin Hall, and Physical Plant (recycled water)
2. Morehead Utility Plant Board (See Diagram 2), collected from:
   a. raw (Licking River)
   b. flashmix
   c. clearwell
   d. storage tanks: U.S. 60 and Divide Hill
   e. points of distribution: MUPB shop, city garage, and private homes
3. Health facilities*, collected from:
   a. St. Claire Medical Center (showers)

Diagram 1. Morehead State University Treatment Process and Points of Distribution*



*Distribution pipe materials include plastic, stainless steel, and cast iron

Diagram 2. Morehead Utility Plant Board Treatment Process and Points of Distribution*



*This system supplies water to Bath, Fleming and Rowan Counties

b. Morehead Life Care Center (showers)

c. Dialysis Clinic (See Diagram 3): pre-purified, and holding tank (purified)

4. Potable (drinking) water (unchlorinated), collected from:

a. drilled well

b. dug wells

c. cisterns

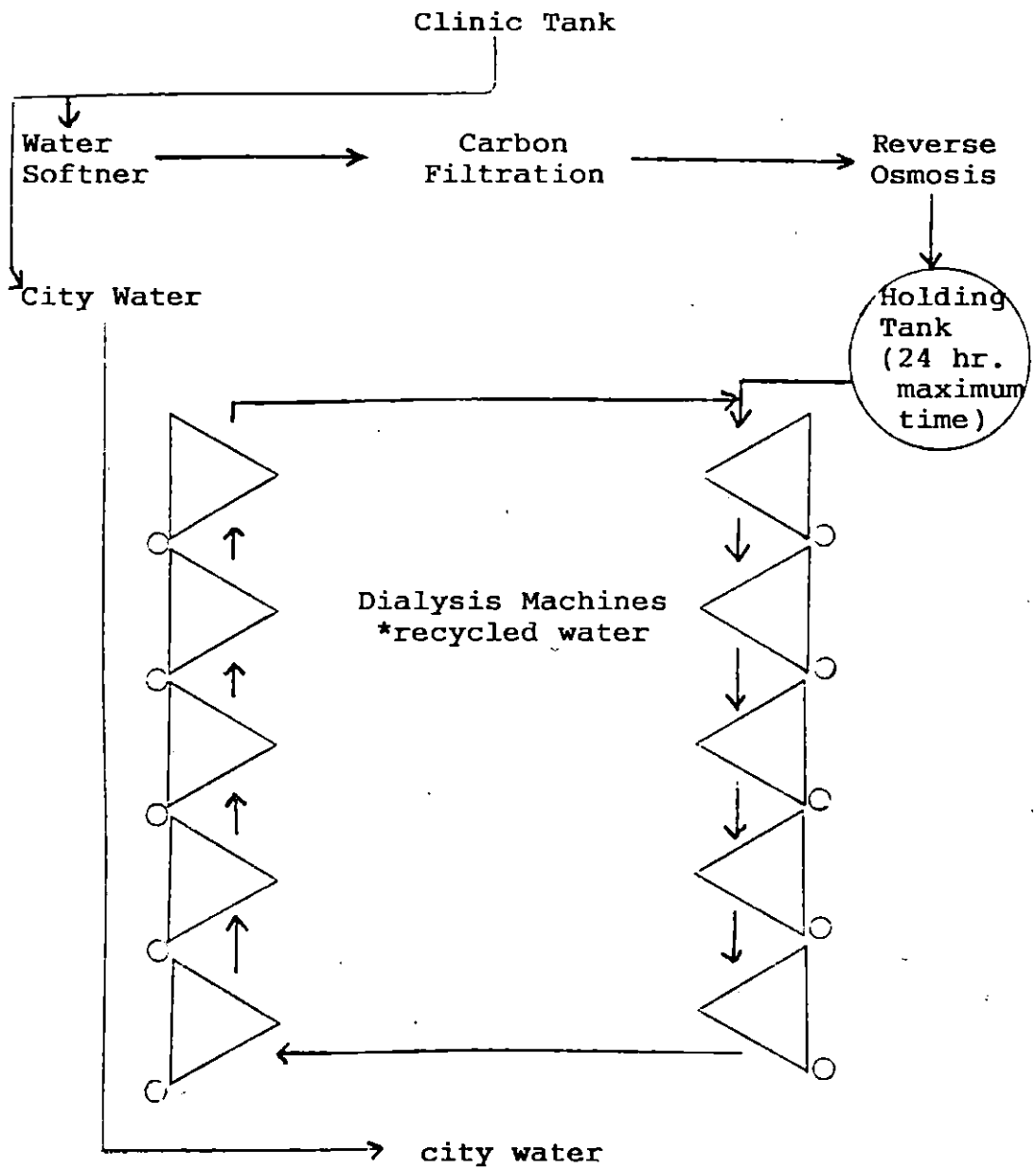
d. springs

Collection of Samples

Tap/faucet collection. Approximately one liter samples were collected from various aquatic habitats in sterile wide-mouth screw-cap plastic bottled (Bel-Art Products, Calvert Industrial Supplies, Calvert City, KY) or one-half gallon polyethylene jugs (Cole-Parmer, Chicago, IL). All bottles were treated with 0.1ml of 10% sodium thiosulfate per 100ml volume and autoclaved. Taps were flushed for two minutes prior to collection, except the MUPB clearwell which maintains constant water flow in the line.

Samples were immediately transported to the laboratory for processing. To prevent exposure to extreme heat, certain samples were transported to the laboratory in insulated containers (Freeze Safe, Polyfoam Parker Corp.,

Diagram 3. Dialysis Clinic Purification Process



Wheeling, IL) with ice. Samples not immediately processed were refrigerated at 4°C for no more than 30 hours.

University raw and clearwell collection: Approximately 1.0ml samples were collected from Triplett Creek and the clearwell by using a sterile glass jar. Each sample was transferred to a one liter wide-mouth, screw-cap plastic bottle for transport to laboratory for processing.

Unchlorinated samples. Due to the location of certain wells, springs, and cisterns, individuals were asked to mail samples to the water testing laboratory. Participants were provided with four 150ml glass screw cap bottles (Calvert Ind., Calvert City, KY), instructions for collection, a data sheet and insulated mailers. These samples were processed immediately upon receipt so as to remain within the 30-hour time limit (American Public Health Association, 1985).

Processing of Samples

Filtration. All samples were processed for fungal isolation and identification, bacterial enumeration and physiochemical analysis (See Figure 1). Depending upon sample type, 10, 25, and 50ml aliquots were concentrated by filtration, using a sterile 47mm filter assembly (Millipore Corporation, Bedford, MA) containing a 0.45um porosity GN-6 filter (Gelman Sciences, Inc., Ann Arbor,

Figure 1. Protocol for Analysis of Samples From a Single Sample Site*

A. Fungal Enumeration

1. 200ml Sample
2. Filtration of appropriate volume
3. Gelman GN-6 filter, 0.45um

B. Isolation Media

1. CZApek-Dox + antibiotics
2. SAB + rose bengal + antibiotics

C. Identification Media

1. SAB-Emmons
2. Malt Extract
3. CZApek-Dox

D. Microculture Technique

A. Bacterial Enumeration

1. 500ml sample
2. Filtration of appropriate volume
3. Millipore, White HA 0.45um
4. m-Endo (TC)

B. Heterotrophic Plate Count

1. R2A

A. Physiochemical Analysis

1. 150ml sample
2. temperature
3. pH
4. turbidity
5. conductivity
6. total chlorine

MI) to recover fungal propagules. All samples were processed in the Germ-Free Bioflow Hood (Germ-Free Laboratories, Miami, FL). After each sample was filtered, the membrane was aseptically removed from the filtration unit. The membrane was placed grid side up onto two plates of Rose Bengal (Rosenzweig, 1986) agar (RBA) and CZApek-dox agar (CZA) (Nagy and Olson, 1982) (Difco Laboratories, Detroit, MI). Refer to Table 4 for RBA composition and Table 5 for CZA composition.

Total coliform enumeration was performed by filtering 100ml samples of water through a 0.45um porosity type HA filter (Millipore Corporation, Bedford, MA). The filter was aseptically removed from the filtration unit and transferred to a 50mm diameter plate (Millipore Corporation, Bedford, MA) containing 2.0ml of m-Endo broth (Difco Laboratories, Detroit, MI).

Mycological. The membrane was aseptically transferred to two plates each of RBA (Rosenzweig, 1986) and CZA (Nagy and Olson, 1982). The RBA and CZA plates were incubated at room temperature (25°C) and scored daily for 7 days (See Figure 2).

Bacteriological. Following filtration for total coliform analysis, the membrane was aseptically removed from the holder and transferred to a 50mm diameter plate (Millipore Corporation, Bedford, MA) containing 2.0ml

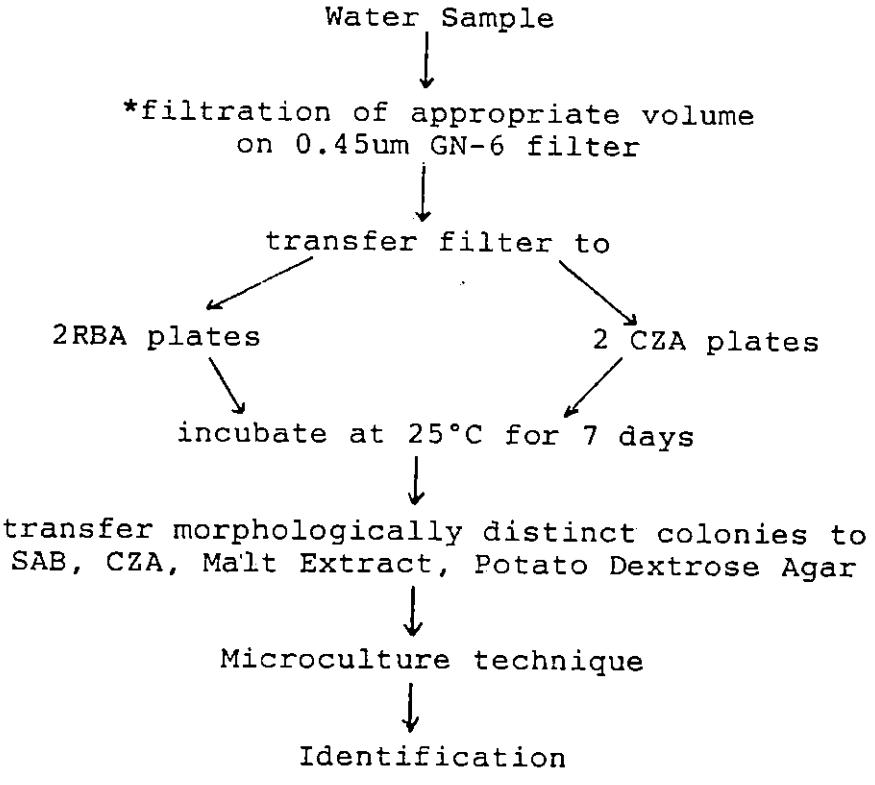
Table 4. Rose Bengal Agar Composition

Type of Constituents	Amount/Liter
Dextrose	40.0gm
Neopeptone	10.0gm
Agar	20.0gm
Rose Bengal	33.0ug
Polymixin B	40,000ug
Penicillin G	20,000U
Streptomycin	80,000ug

Table 5. CZApek-Dox Agar Composition

Type of Constituents	Amount/Liter
Sucrose	30.0gm
Sodium Nitrate	3.0gm
Dipotassium phosphate	1.0gm
Magnesium sulfate	0.5gm
Potassium chloride	0.5gm
Ferrous sulfate	0.01gm
Agar	15.0gm
Polymixin B	40,000ug
Penicillin G	20,000U
Streptomycin	80,000ug

Figure 2. Protocol for Isolation, Enumeration and Identification of Fungi from Water Samples.



*Volumes filtered: raw water - 10ml
treated water - 50ml

of m-Endo broth (Difco Laboratories, Detroit, MI). Total coliforms exhibited a metallic sheen. Heterotrophic plate counts (HPC's) were enumerated by pour plate techniques on low nutrient agar, R2A (Nagy and Kelly, 1982), and incubated at room temperature (25°C) for 7 days. Total colonies (pigmented and non-pigmented) were enumerated by use of a Quebec Colony Counter (American Optical, Buffalo, NY). Tests were performed in duplicate with inocula of 1.0ml and 0.1ml for chlorinated waters and 0.1ml and 0.01ml for raw waters (See Figure 3).

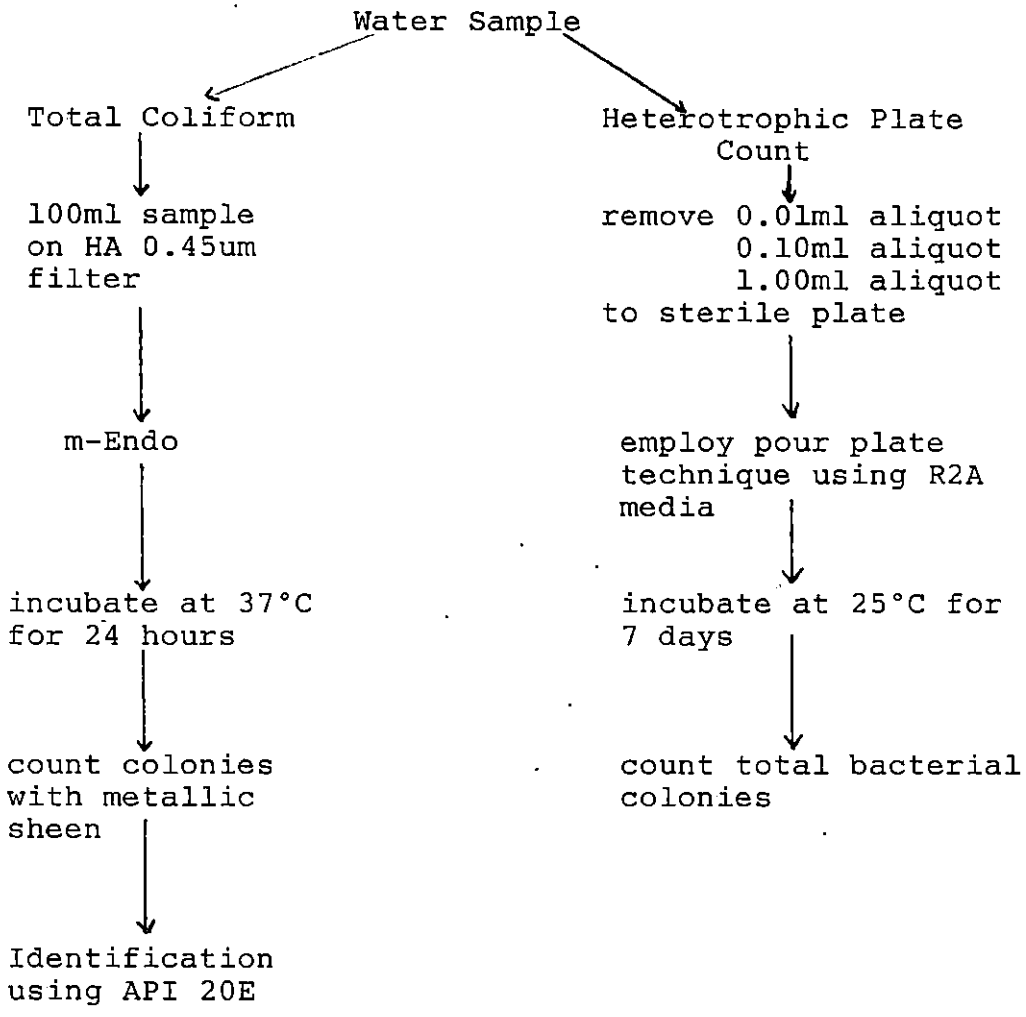
Physiochemical Analysis

Prior to mycological and bacteriological analyses the following physiochemical parameters were measured: 1) temperature, 2) pH, 3) residual chlorine and 4) turbidity. Temperature and pH were measured using a Markson Model 90 pH/temperature meter (Markson, Phoenix, AZ). Residual chlorine was measured using the HACH DPD method (HACH Company, Loveland, CO). Turbidity levels were determined for each sample using a Nephelometric Turbidimeter (HF Instruments Limited, Ontarion, Canada).

Preparation of Rose Bengal Agar

The preparation of Rose Bengal Agar (RBA) used to isolate fungi from all samples was prepared by utilizing

Figure 3. Protocol for the Isolation and Enumeration of Bacteria From Water Samples



Sabouraud dextrose agar (Difco Laboratories, Detroit, MI) supplemented with 33.3ug/ml of Rose Bengal (Sigma Chemical Company, St. Louis, MI). The media was autoclaved at 121°C for 15 minutes. It was then placed into a 50°C water bath and cooled prior to the addition of antibiotics: 1) Polymixin B (Sigma Chemical Company, St. Louis, MI) at 40ug/ml, 2) Penicillin G (Pfizer Inc., New York, NY) at 20units/ml, and 3) Streptomycin (Pfizer Inc., New York, NY) at 80ug/ml. The media was dispensed (40ml/plate) into sterile 15 x 100mm petri dishes (1029 Petri Dish, American Scientific Products, Cleveland, OH) under the Germ-free bioflow hood.

Identification

Morphologically distinct colonies isolated from each sample were transferred to a battery of identification media (Sabouraud, CZApek-dox, Malt Extract, and Potato Dextrose Agar). Microcultures and lactophenol cotton blue (LPCB) wet mounts were prepared to observe reproductive structures. Based on macroscopic colony characteristics and asexual or sexual reproductive structures, isolates were identified to genus using the following keys/references:

1) Barnett and Hunter. 1987. Illustrated Genera of Imperfect Fungi.

2) Beneke and Rodgers. 1980. Medical Mycology Manual.

3) Domsch, Gams, and Anderson. 1980. Compendium of Soil Fungi.

4) Emmons et al. 1982. Medical Mycology.

5) Gilman, Joseph. 1957. The Manual of Soil Fungi.

Colonies lacking distinguishable reproductive structures were not identified.

Quality Assurance

Preparation of filtration area. The bioflow hood, work area bench tops, and floors were disinfected with a Wescodyne solution (West Chemical Products, New York, NY) approximatley one-half hour prior to processing of samples. The air in the work area was ozone purified using a Clinic-Aire purifier (Aqua-Mist, Inc., Winston-Salem, NC).

Air controls. Two inoculated plates (one each of CZApek-dox and RBA) were opened in the bioflow hood during filtration to check for possible air contamination.

Sterile water controls. Sterile water was used as a control. Control samples were employed at the beginning and end of each series of processed samples. Control samples were treated in the same manner as samples intended for mycological and bacteriological analyses.

CHAPTER IV

RESULTS AND DISCUSSION

One hundred and twenty-one water samples were collected and processed from two distribution systems, a hemodialysis purification system and selected unchlorinated waters in an attempt to demonstrate the presence of filamentous fungi. A membrane filtration technique using RBA and CZA media was utilized for fungal recovery. Bacteriological and physiochemical parameters were also examined to determine if fungal presence can be correlated with these parameters.

Of the 121 samples collected, 66% (80 of 121) were positive for filamentous fungi. The majority (67%) of positive samples contained more than one fungal isolate. The mean CFU for chlorinated and unchlorinated samples were 17.25 and 148.2, respectively. The CFU range was 0.5 to 425.0 for chlorinated samples and 0.5 to 1265 for unchlorinated samples. In chlorinated samples the most prevalent identified fungal genera recovered were Acremonium (17.0%), Cladosporium (15.5%), Fusarium (15.1%), and Aspergillus (8.5%); in unchlorinated waters Acremonium (14.8%), Trichoderma (13.4%), Fusarium (8.3%) and Cladosporium (8.3%) were the identified fungi most frequently observed. The percentage frequency of genera

are listed in Figure 4 for chlorinated samples and Figure 5 for unchlorinated samples.

Acremonium (20.3%), Cladosporium (19.8%), and Exophiala (11.5%) exhibited the highest percentage of total CFU's in chlorinated waters. The most numerous genera isolated from untreated water samples included Phoma (25.6%), Phialophora (11.1%), and Geotrichum (10.8%). See Figure 6 for total CFU percentages for chlorinated samples and Figure 7 for unchlorinated samples.

Morehead State University Water System

Twenty-four of the 121 samples were collected from a small university distribution system (See Diagram 1). Four samples were collected from Triplett Creek and an average CFU of 335 per 100ml sample was determined. The range for raw samples was from 50 to 1045 CFU's. Refer to Table 6 for the specific genera isolated.

Of those samples obtained from the clearwell and distribution taps, 45% (11 of 24) were positive for filamentous fungi. The range of positive MSU samples within the distribution system was from 0.5 to 47 CFU. Table 7 shows the different parts of the system sampled, percentage of positive samples and the average CFU. Cladosporium (34.5%), Fusarium (10.3%), and Aspergillus (10.3%) were the most frequent genera isolated, as well

Figure 4. Mean percentage frequencies of filamentous fungal genera from chlorinated samples.

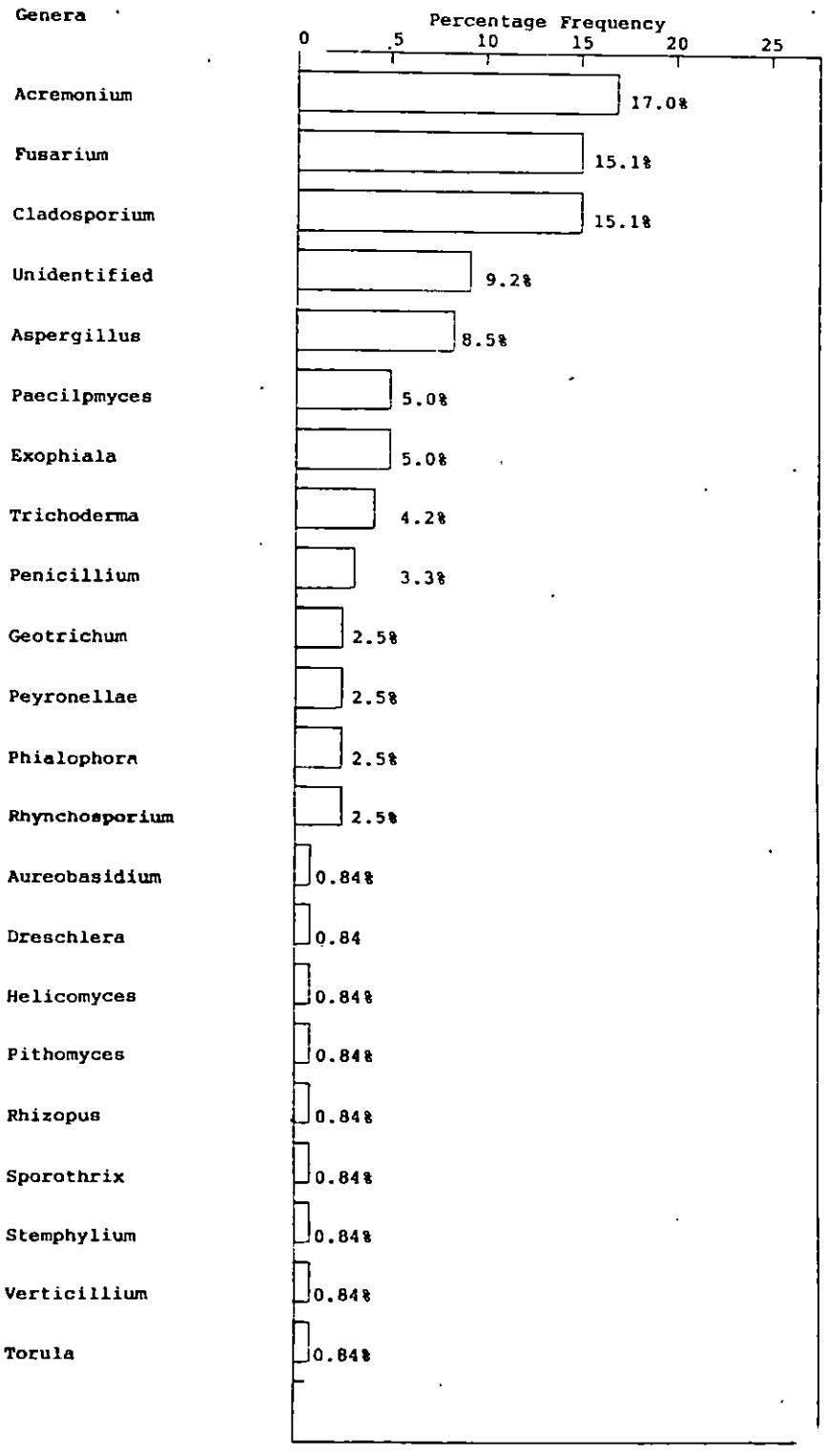


Figure 5. Mean percentage frequencies of filamentous fungal genera isolated from unchlorinated samples.

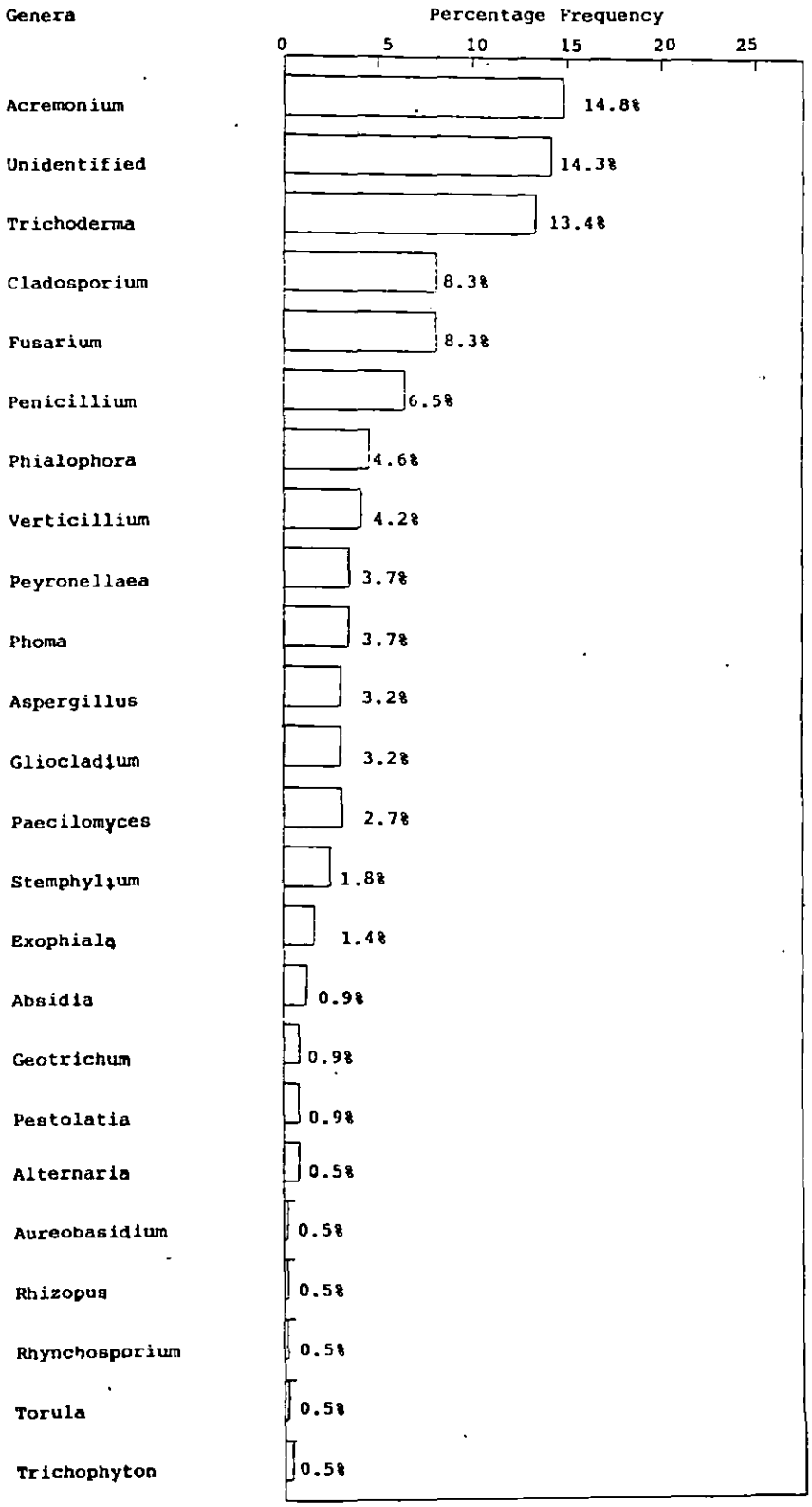


Figure 6. Mean percentage total CFU's of filamentous fungal genera isolated from chlorinated samples.

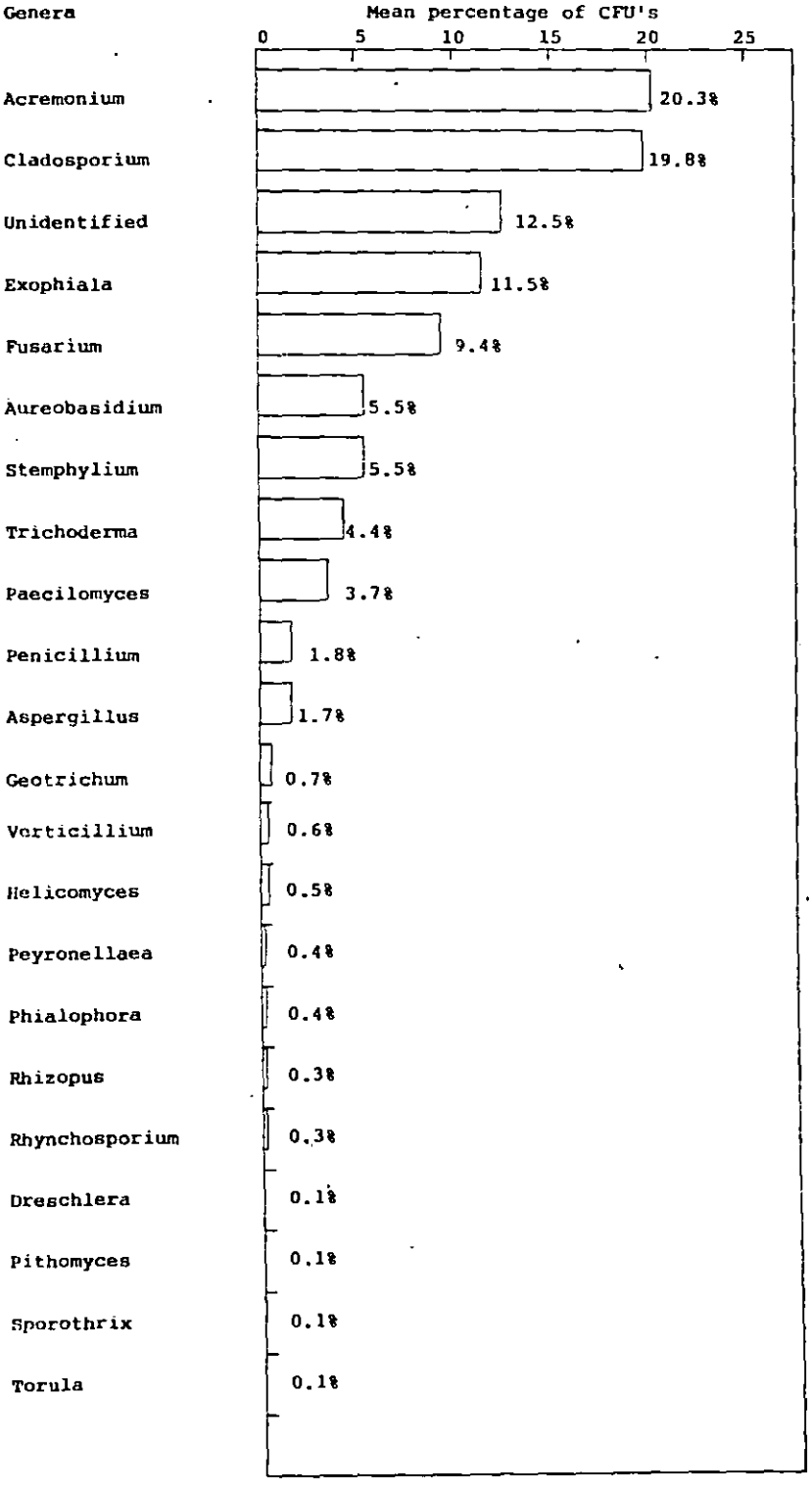


Figure 7. Mean percentage total CFU's of filamentous fungal genera isolated from unchlorinated samples.

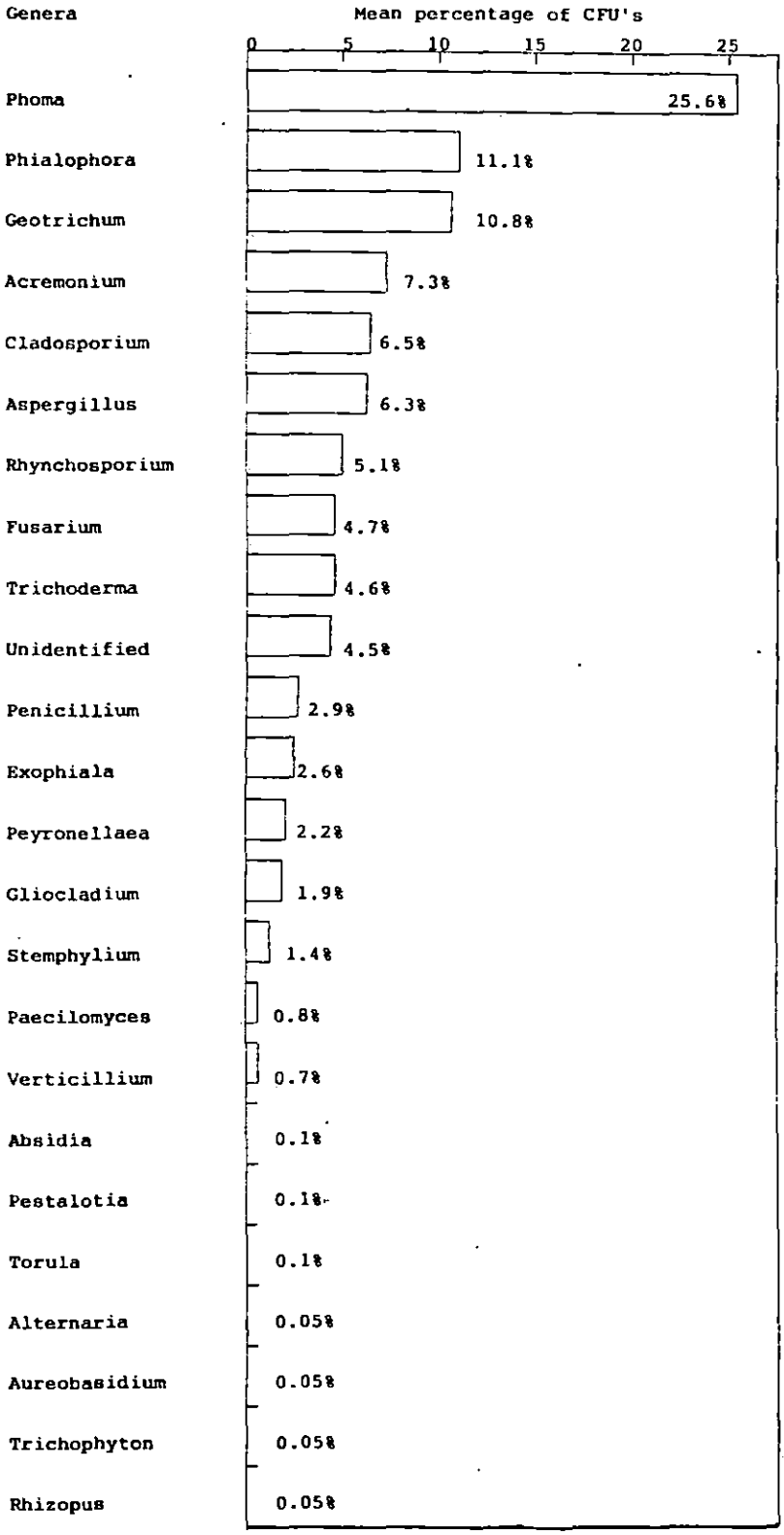


TABLE 6

Filamentous Fungal Genera Identified From Triplett Creek and Morehead State University Distribution System

Genera	October CFU	November CFU	January CFU	February CFU	#/100ml CFU	#+ CFU
RAW						
Absidia	0	0	3	0	0.75	3
Acremonium	25	0	13	10	12	16
Aspergillus	250	0	0	43	73	147
Aureobasidium	0	0	3	0	0.75	3
Cladosporium	125	35	0	35	49	65
Fusarium	0	55	8	23	22	29
Geotrichum	525	0	0	0	131	525
Paecilomyces	0	0	10	28	10	19
Penicillium	50	0	0	0	12	50
Peyronellaea	0	0	0	25	6	25
Phoma	50	0	5	0	14	28
RAW						
Rhynchosporium	250	0	0	0	62	250
Torula	0	0	8	0	2	8
Trichoderma	0	8	5	0	3	7
Trichophyton	0	0	3	0	0.75	3
CLEARWELL						
Aureobasidium	25	0	0	0	6	25
Cladosporium	28	0	0	0	7	28
Fusarium	0	0	11	0	3	11
Geotrichum	3	0	0	0	0.75	3
Helicomyces	3	0	0	0	0.75	3
Sporothrix	0	0	1	0	0.25	1
Stemphylium	25	0	0	0	6	25

Genera	October	November	January	February	#/100ml	#+
TANK						
Fusarium	0	0	10	0	2.5	10
MIGNON TOWER						
Cladosporium	1	3	33	10	12	12
Geotrichum	1	0	0	0	0.25	1
Penicillium	1	0	0	0	0.25	1
Phialophora	0	0	0	1	0.25	1
CARTMELL HALL						
Aspergillus	1	0	0	0	0.25	1
LAPPIN HALL						
Rhizopus	0	2	0	0	0.5	2
Aspergillus	1	0	0	0	0.25	1
PHYSICAL PLANT						
Fusarium	0	0	11	0	3	11

TABLE 7

Percent of Samples Positive for Fungi
at Various Points in the MSU
Distribution System

Sampling Point	Number of Samples	Percent Positive	CFU $\bar{x}$ for Samples +
Clearwell	4	50%	64.8/ 11.5*
Distribution Taps	16	50%	9.7
Storage Tank	4	25%	10.0
Raw (Triplett Creek)	4	100%	335

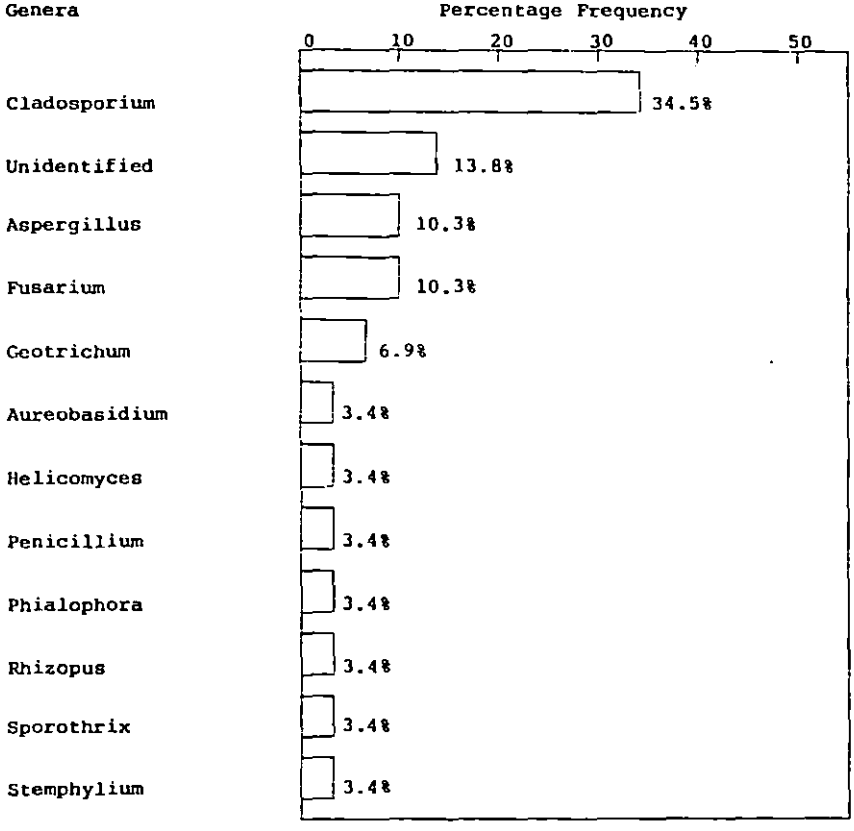
*Mean CFU for clearwell excluding October data

as the majority of total CFU's (See Figure 8).

October and January clearwell samples were positive for filamentous fungi with an average of 64.8 CFU. The results were disproportionately high due to the October CFU (118) as compared to a mean CFU for November (0.0), January (11.5) and February (0.0). During the week of the October clearwell sample collection, the MSU distribution system was experiencing taste and odor problems due to the presence of algae and the chlorine/ algae interaction producing trihalomethanes (THM) (Hilderbrand, 1986). THM, a carcinogen, is formed during the chlorination of waters which contain precursor compounds, mostly humic substances (Amy, Chadik, and Choudhury, 1987). The abundance of fungal growth in the October sample was attributed to low chlorine levels in the system due to the excessive THM formation. Five of the seven genera isolated in October were not recovered in the remaining three monthly clearwell samples (See Table 6). Excluding the October data, the CFU average for the remaining three samples was 3.8/100ml, indicating a dramatic decrease (97%) from source water (335.0 CFU).

Once the water left the clearwell an increase in filamentous fungi was observed within the distribution system. An average of 5.0 fungal propagules/100ml was observed. Fifty percent of the 16 samples collected

Figure 8. Mean percentage frequencies of filamentous fungal genera from Morehead State University clearwell and distribution system.



from the distribution taps at MSU were positive for filamentous fungi, with each positive site contributing at least one positive sample during the collection period. The mean CFU of all positive samples was 9.7, while the overall mean for MSU distribution taps was 5.0 CFU. See Table 6 for specific genera and mean CFU for each MSU collection site.

Samples collected from Mignon Tower had recovery values higher than any other MSU distribution site. All four samples collected from this site were positive for filamentous fungi with Cladosporium spp. being the dominant isolate. Cladosporium was not found at any other site within the MSU distribution system, but did occur in both raw and clearwell samples during the collection period.

Of the four samples collected from the MSU storage tank only one was positive. Only Fusarium was isolated from the positive sample with a mean CFU of 10.0. The average CFU for the four MSU storage tank samples collected was 2.5. Table 7 summarizes these findings.

Morehead Utility Plant Board

Sixty-four of the 121 samples collected were taken from the Morehead Utility Plant Board (MUPB) water treatment plant and distribution system (See Diagram

2). Four of the 64 samples were collected from the Licking River (raw water). These four samples were positive for filamentous fungi with an average of 42.7 CFU/100ml. The range was from 16.7 to 105.0 fungal propagules per 100ml, with the October sampling yielding the highest CFU's during the sampling period. Specific genera isolated are listed in Table 8.

All four flashmix samples were positive, with a mean CFU of 8.9/100ml. This value shows a significant decrease in filamentous fungal recovery values compared to the CFU of the raw water source. A range of 2.0 to 25.0 CFU's was observed, with the greatest recovery occurring in the February sample.

Of the four clearwell samples assayed, only the November sample was positive, with Fusarium being isolated. The low CFU's observed in clearwell samples (1.0) when compared to raw (42.7) and flashmix (8.9) samples reflects successful fungi reduction by the treatment process.

Forty-four percent of samples collected from distribution taps were positive for filamentous fungi. CFU's ranged from 0.5 to 12.5 with an average of 2.3 CFU for positive samples and 0.92 for all samples.

Seven of the eight collected from MUPB storage tanks were positive for filamentous fungi. A mean CFU for these samples was 3.7 with a range of 0.5 to 9.6 CFU's.

TABLE 8

Filamentous Fungal Genera Identified From
Licking River, Flashmix, and Points of Distribution
City of Morehead

Genera	October CFU	November CFU	January CFU	February CFU	X/100ml CFU	X+ CFU
RAW						
Acremonium	10	2	0	43	14	18
Fusarium	45	0	0	30	19	36
Paecilomyces	0	0	0	3	0.75	3
Pestalotia	0	0	0	8	2	8
Penicillium	0	2	3	3	2	3
Peyronellaea	0	0	3	0	0.75	3
Phialophora	0	0	3	0	0.75	3
Phoma	0	0	3	0	0.75	3
Stemphylium	0	0	0	23	6	23
Trichoderma	40	10	0	33	22	28
Verticillium	13	3	3	0	5	6
FLASHMIX						
Acremonium	1	1	6	5	3	3
Aspergillus	1	0	0	0	0.25	1
Dreschslera	1	0	0	0	0.25	1
Fusarium	0	1	1	0	0.25	1
Rhynchosporium	1	0	0	0	0.25	1
Pithomyces	1	0	0	0	0.25	1
Trichoderma	0	0	0	18	5	18
Verticillium	0	0	0	3	0.75	3
CLEARWELL						
Fusarium	0	1	0	0	0.25	1

Genera	October CFU	November CFU	January CFU	February CFU	x/100ml CFU	x+ CFU
MUPB SHOP						
Acremonium	4	0	0	0	1	4
Aspergillus	3	0	0	0	0.75	3
Cladosporium	2	1	12	1	4	4
Fusarium	0	5	0	0	1	5
Peyronellaea	0	0	2	0	0.5	2
Phialophora	0	0	1	0	0.25	1
CLEARFIELD						
Acremonium	1	0	1	0	0.5	1
Fusarium	0	0	0	1	0.25	1
Peyronellaea	0	0	0	1	0.25	1
Phialophora	0	0	0	1	0.25	1
CITY GARAGE						
Aspergillus	0	1	0	0	0.25	1
ST. CLAIRE #1						
Acremonium	1	0	0	0	0.25	1
Penicillium	1	0	0	0	0.25	1
ST. CLAIRE #2						
Cladosporium	1	0	0	0	0.25	1
LIFE CARE #1						
Acremonium	0	0	1	1	0.5	1
LIFE CARE #2						
Aspergillus	1	0	0	0	0.25	1
HOLIDAY ESTATES						
Fusarium	0	1	0	0	0.25	1
Geotrichum	1	0	0	0	0.25	1

Genera	October CFU	November CFU	January CFU	February CFU	x/100ml CFU	x+ CFU
US 60 TANK						
Acremonium	1	0	2	0	0.75	2
Cladosporium	1	0	1	0	0.5	1
Fusarium	0	1	0	0	0.25	1
Rhynchosporium	0	0	1	0	0.25	1
Paecilomyces	2	0	0	0	0.5	2
Trichoderma	2	0	0	0	0.5	2
DIVIDE HILL TANK						
Acremonium	1	0	0	0	0.25	1
Aspergillus	1	2	0	0	0.75	2
Fusarium	0	3	0	0	0.75	2
Penicillium	6	0	0	0	1.5	6
Trichoderma	1	1	0	0	0.5	1

An evaluation of Table 9 shows that fifty-three percent (32 of 60) of the chlorinated samples (flashmix, clearwell, tanks, and distribution taps) were positive for filamentous fungi. Fusarium and Acremonium were the most frequent genera isolated (See Figure 9). Fusarium was recovered from the raw and flashmix samples and five sites within the distribution system. A comparison of the fungal recovery in both treatment systems is summarized in Figure 10.

Unchlorinated Waters

Twenty-five samples were collected from ground water (dug wells, drilled wells, and springs) and cisterns in eastern Kentucky. Ninety-two percent (23) of the unchlorinated samples were positive for filamentous fungi. Table 10 lists the fifteen genera isolated from unchlorinated waters. Acremonium (15.5%) and Trichoderma (14.2%) were the most frequently isolated (See Figure 11). However, Phoma (39.5%) and Philaphora (18.0%) demonstrated the highest percentage of the total CFU's observed (See Figure 12).

Dug Wells

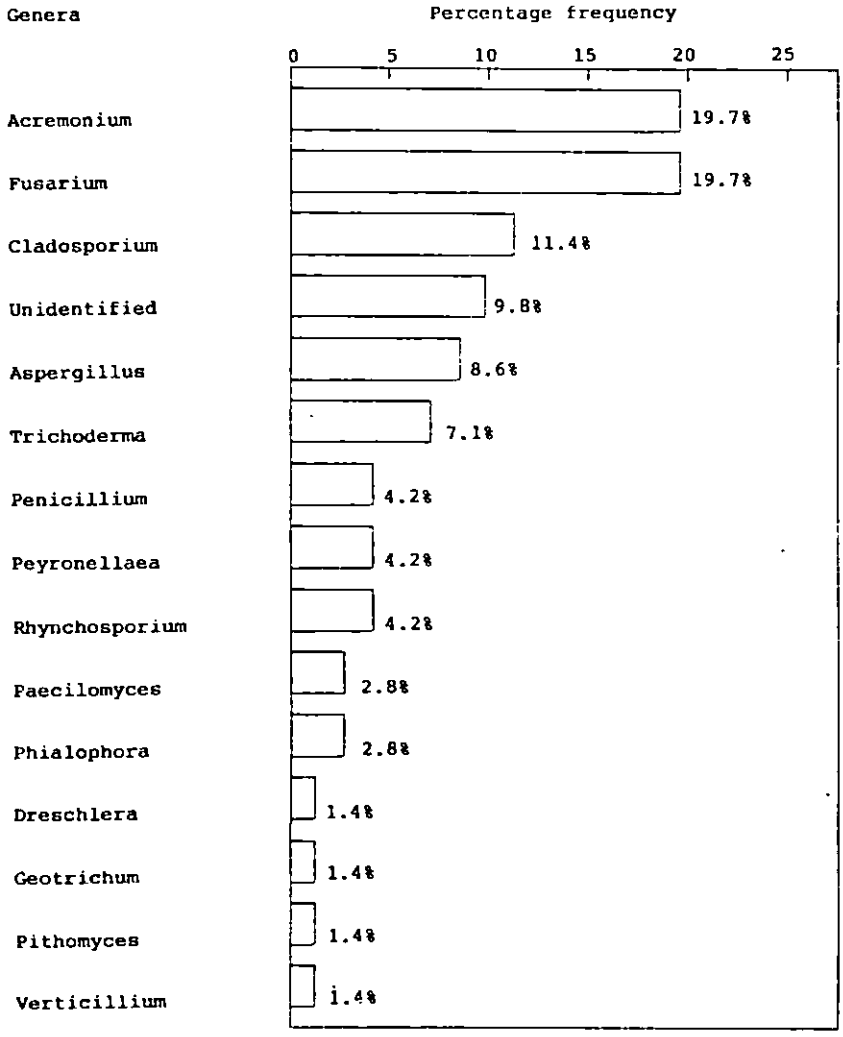
Data from six samples collected from dug wells are depicted in Figure 13 and 14. All open air dug well

TABLE 9

Percent of Samples Positive for Fungi
at Various Points in the City of Morehead
Distribution System

Sampling Point	Number of Samples	Percent Positive	CFU $\bar{x}$ for Samples +
Raw	4	100%	42.7
Flashmix	4	100%	8.9
Clearwell	4	25%	1
Distribution Taps	44	45%	2.3
Storage Tanks	8	88%	3.0

Figure 9. Mean percentage frequency of filamentous fungal genera in City of Morehead chlorinated samples.



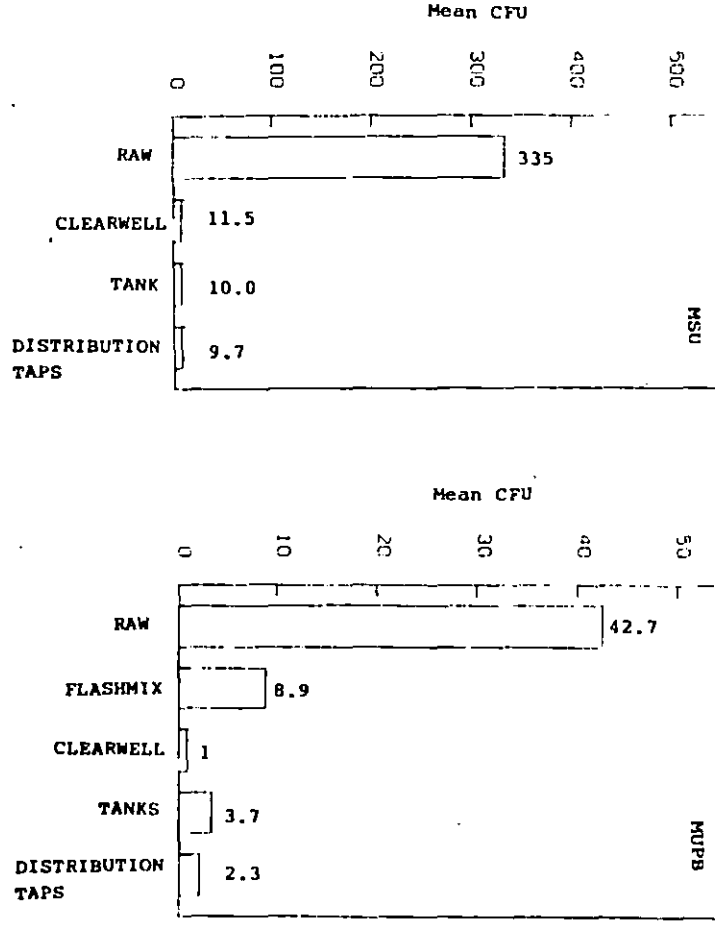


Figure 10. Comparison of MSU and MUPB Treatment and Distribution Systems.

TABLE 10

Isolation Sites of Identifiable Filamentous Fungi in Unchlorinated Water

Genera	Dug Wells	Springs	Cisterns	Drilled Well
Absidia			X	
Acremonium	X	X	X	X
Alternaria			X	
Exophiala		X		
Fusarium	X	X	X	
Gliocladium	X		X	
Penicillium		X	X	
Peyronellaea	X	X	X	
Phialophora		X	X	X
Phoma		X	X	
Rhizopus		X		
Stemphylium			X	
Trichoderma	X	X	X	
Verticillium		X		

Figure 11. Mean percentage frequency of filamentous fungal genera isolated from spring, wells, and cistern samples.

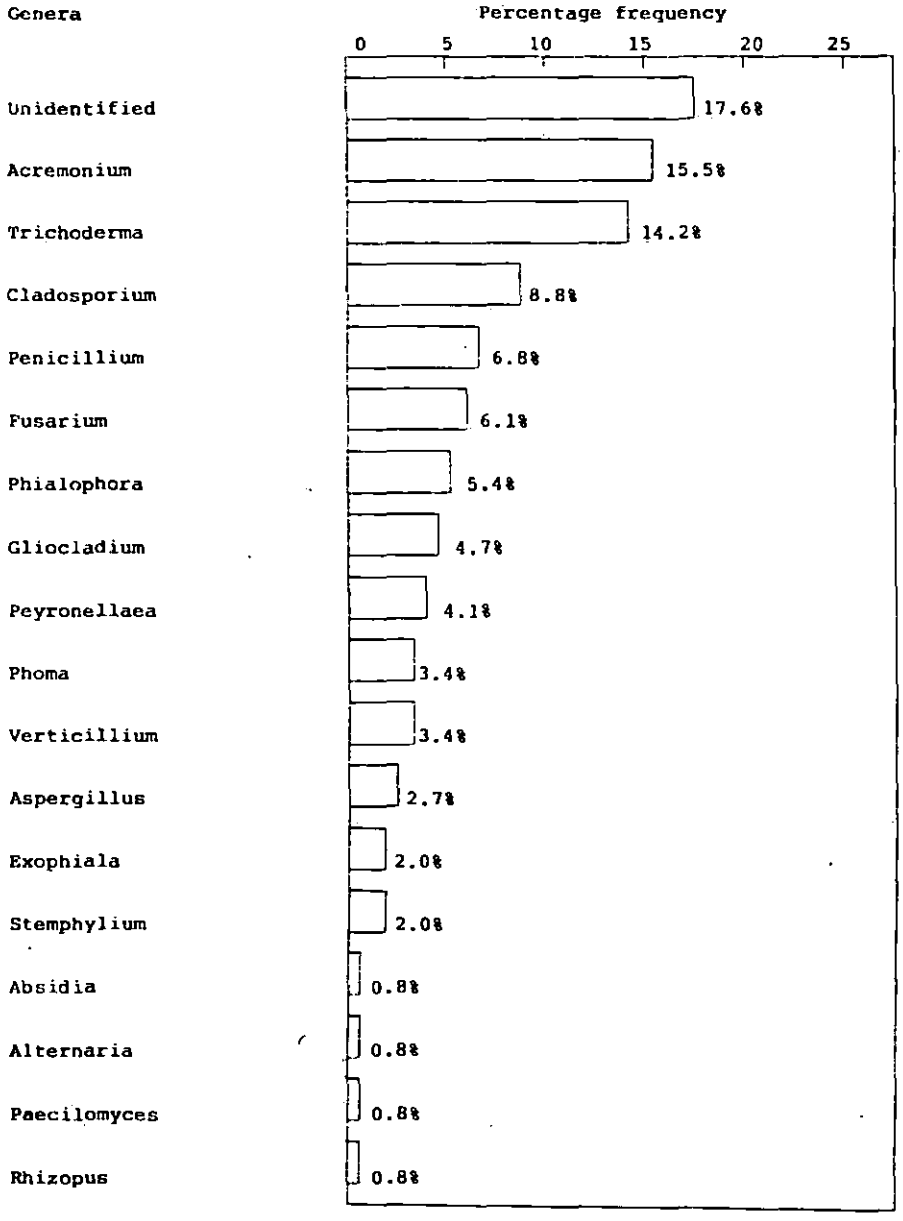


Figure 12. Mean percentage of total CFU's of filamentous fungal genera isolated from spring, wells, and cistern samples.

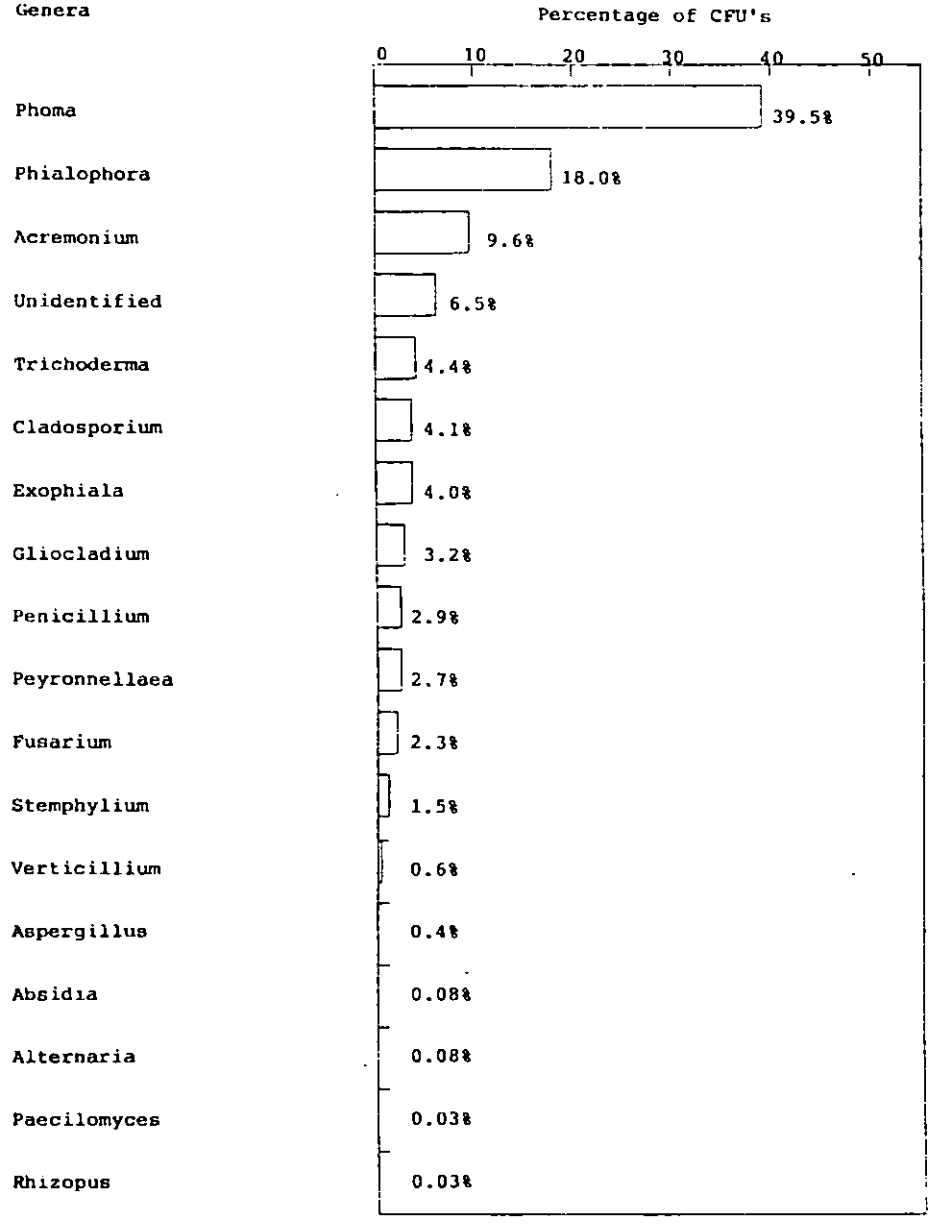


Figure 13. Percentage frequency of Fungi Isolated From Dug Wells.

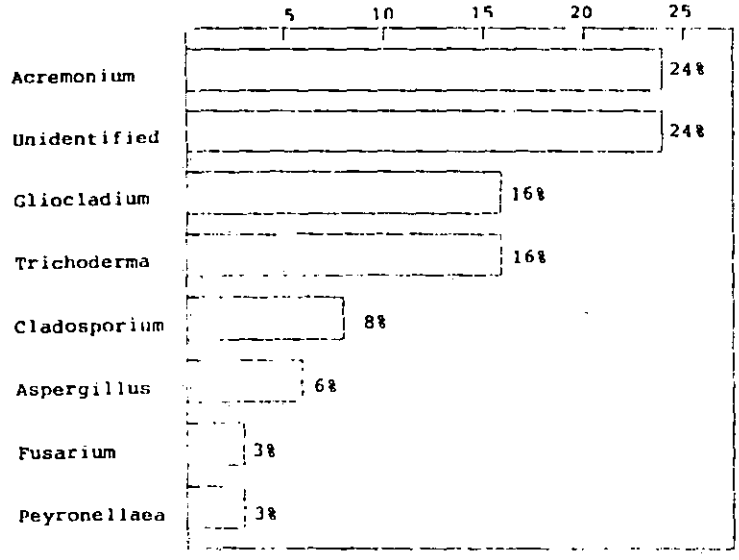
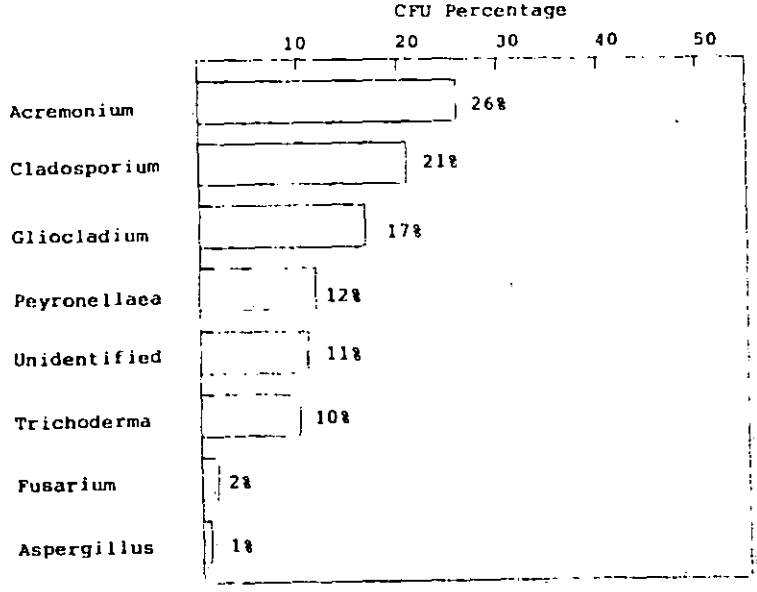


Figure 14. Percentage CFU of Fungi Isolated from Dug Wells.



samples were positive for filamentous fungi and recovery values ranged from 17.5 to 167.5 fungal propagules/100ml with a mean CFU of 78.1. Seven genera were recovered from dug wells with Acremonium the most frequent isolate, and the genus with the greatest percentage of total CFU's (25.5%).

Springs

Eight samples collected from open air springs were all positive for filamentous fungi (See Figure 15 and 16). A mean CFU of 56.0 was determined, with CFU's ranging from 10.0 to 200.0. Genera most frequently isolated included Trichoderma (17.2%) and Acremonium (13.8%). Genera contributing the highest CFU's were Exophiala (27.5%) and Penicillium (18.9%).

Cisterns

Data for cisterns are presented in Figures 17 and 18. All seven cistern samples were positive for filamentous fungi; they had a mean CFU of 291.0, with the mean CFU's ranging from 0.5 to 1265.0. The most frequently isolated genera included Cladosporium (12.2%) and Fusarium (12.2%). Eighty-three percent of the total CFU's observed were Phoma and Phialophora.

Figure 15. Percentage Frequency of Fungi Isolated from Springs.

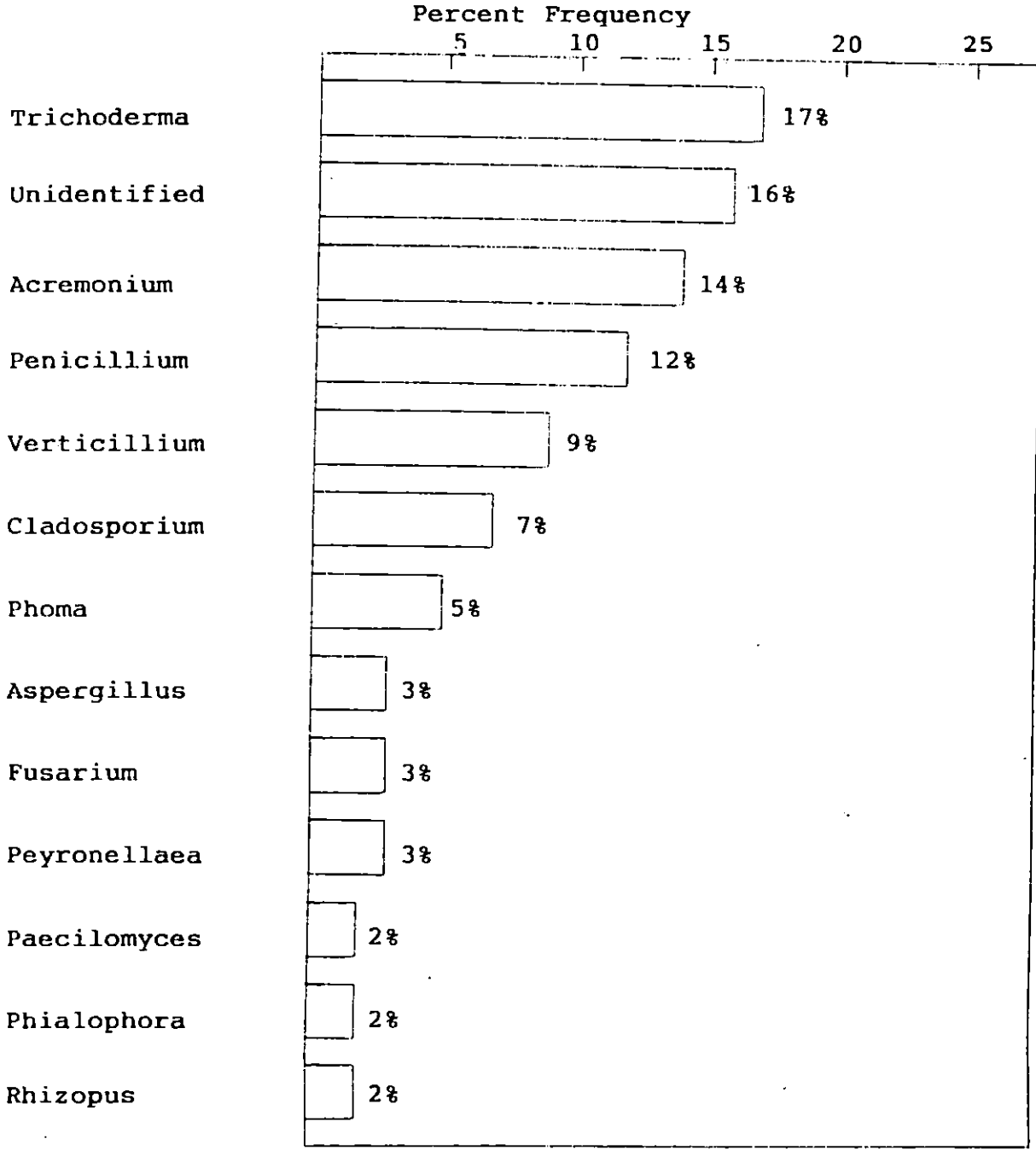


Figure 16. Percentage CFU of Fungi Isolated from Springs.

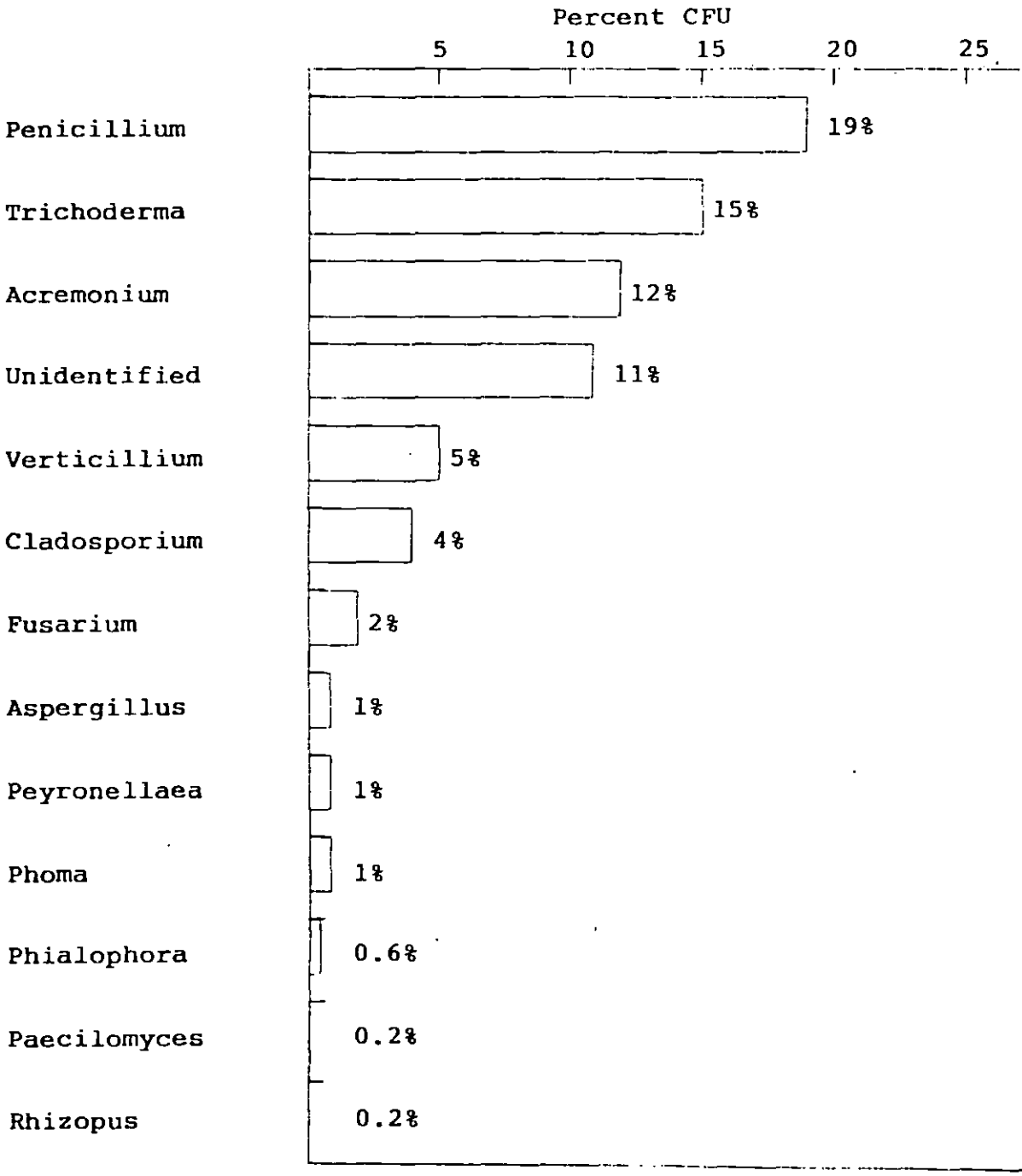


Figure 17. Percentage Frequency of Fungi Isolated from Cisterns.

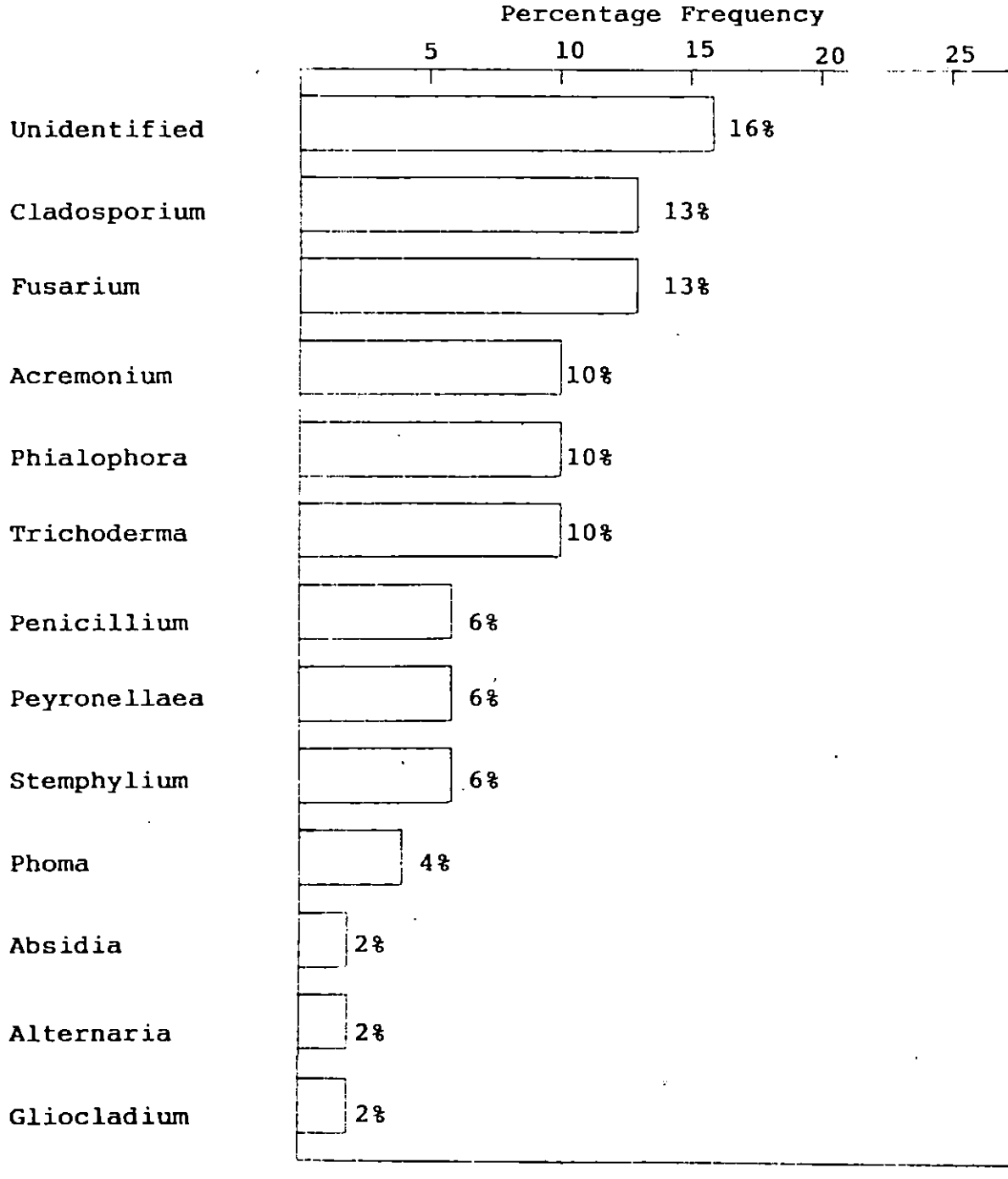
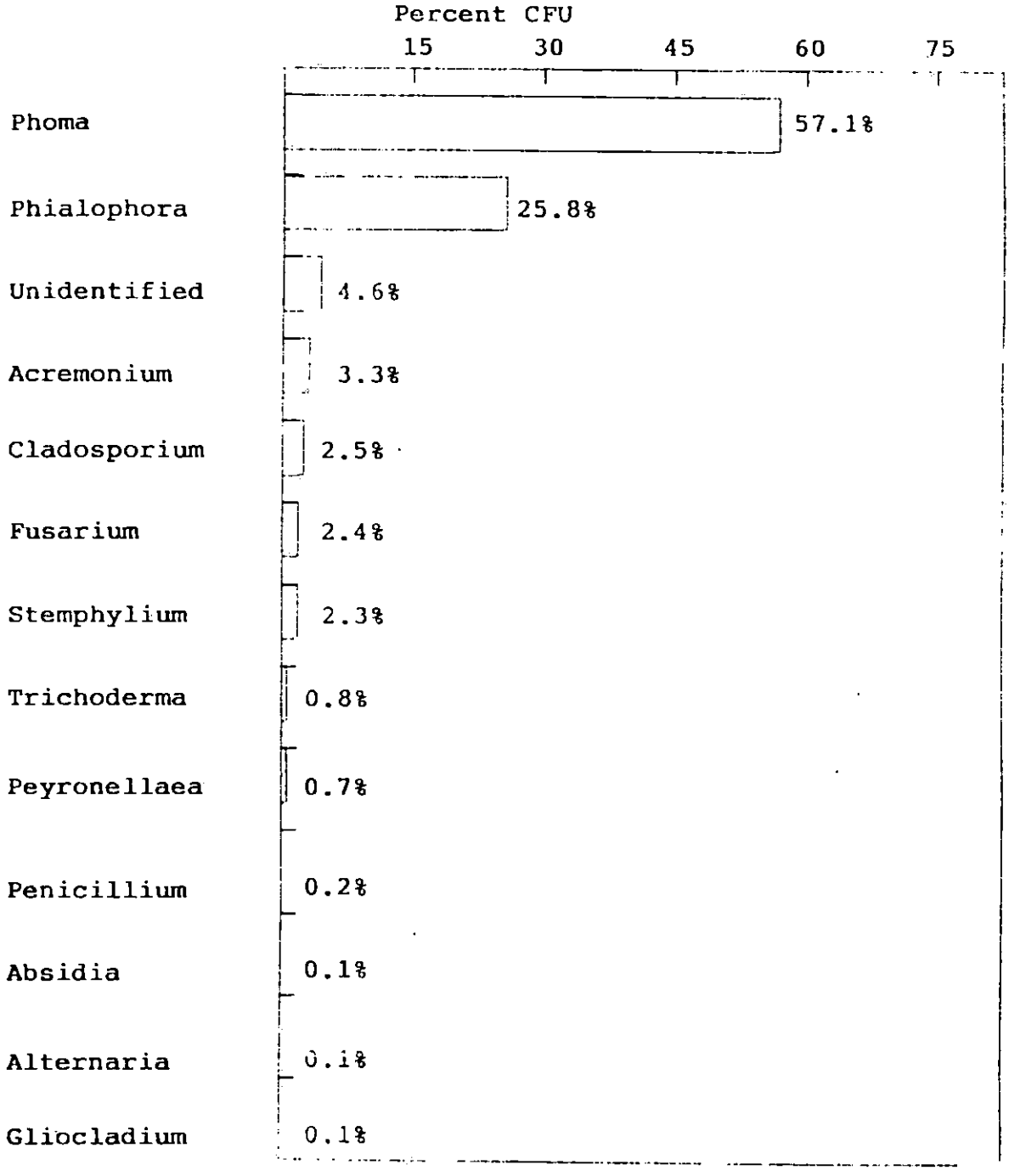


Figure 18. Percentage CFU of Fungi Isolated from Cisterns



Drilled Wells

Two of the four drilled well samples, taken in November and January, were positive for fungi. The mean CFU for positive samples was 3.2 fungal propagules/100ml. The only two genera isolated from drilled wells were Acremonium and Phialophora. Phialophora was isolated from both samples using CZA media, whereas, Acremonium was isolated only in the November sample on the RBA media.

Dialysis Purification System

Eight water samples were collected from the hemodialysis water purification system four prior to purification and four after. Of the four samples collected prior to treatment, the November and January samples demonstrated Fusarium. Both positive samples demonstrated a mean CFU of 1.0 fungal propagules/100ml. However, all four post-purification holding tank samples showed a significant CFU increase in filamentous fungi (35.5); high yeast (283.3) and bacterial populations (432,700) were also observed. An increase in fungal recovery was noted from the October (1.5) to November (47.0) to Janaury (79.0) samples. After notification, the clinic personnel chlorinated the holding tank for 24 hours and flushed the system prior to the collection of the February sample. Fungal recovery was reduced by 80%; bacterial populations

were reduced by 41%, but were not eradicated. Acremonium and Exophiala were the most frequent isolates and had the highest percentage of CFU's observed (See Figure 19 and 20).

Comparison of Isolation Media

An isolation media comparison revealed that RBA demonstrated higher recovery values (57%) than CZA (43%). RBA demonstrated a shorter incubation period (2-3 days) at 25°C, whereas noticeable growth was not observed until the fourth and fifth days of incubation on CZApek-dox agar. RBA recovered all genera isolated in this study except Helicomyces; whereas, CZApek-dox did not recover Absidia, Alternaria, Dreschlera, Pithomyces, Sporothrix, and Trichophyton. RBA is a higher nutrient medium than CZApek-dox. CZApek-dox closely resembles nutritional availability in water distribution lines (Nagy and Kelly, 1982). Table 11 summarizes RBA/CZA recovery values. See Table 12 for a listing of the most common isolates and media type in this study as compared to those of other researchers.

Figure 19. Percentage Frequency of Fungi Isolated from Dialysis Clinic Holding Tank.

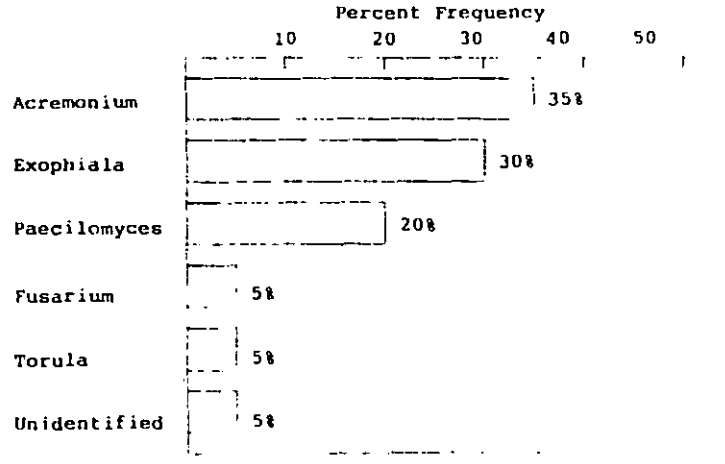


Figure 20. Percentage CFU of Fungi Isolated from Dialysis Clinic Holding Tank.

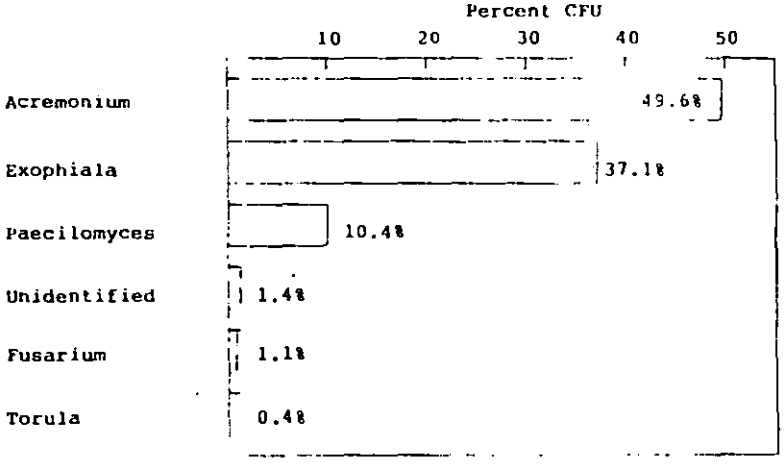


Table 11. Recovery Values of Isolates on Rose Bengal Agar and CZApek-Dox Agar

Rose Bengal Agar	CFU/100ml	CZApek-Dox Agar	CFU/100ml
Geotrichium	1007	Phoma	1820
Phoma	662	Phialophora	786
Acremonium	624	Rhynchosporium	501
Aspergillus	554	Cladosporium	350
Cladosporium	454	Exophiala	285
Fusarium	394	Acremonium	274
Phialophora	304	Trichoderma	204
Trichoderma	299	Peyronellae	177
Stemphylium	145	Penicillium	164
Penicillium	140	Fusarium	149
Gliocladium	75	Gliocladium	120
Peyronellaea	43	Paecilomyces	75
Verticillium	42	Aspergillus	73
Exophiala	18	Geotrichium	50
Torula	15	Aureobasidium	50
Pestalotia	10	Stemphylium	45
Absidia	10	Verticillium	37
Alternaria	5	Helicomyces	5
Aureobasidium	5	Pestalotia	5
Trichophyton	5	Rhizopus	2
Rhizopus	3	Torula	1
Rhynchosporium	2		
Dreschlera	1		
Pithomyces	1		
Sporothrix	1		

CHAPTER V

CONCLUSION

The study indicates that filamentous fungi were present in raw water, can survive present day treatment processes, and may be isolated from various parts of the distribution system. The average number of filamentous fungi propagules for positive chlorinated samples was 17.2/100ml; an average of 148.0 propagules/100ml was observed in positive unchlorinated samples. These results are in general agreement with those reported by Nagy and Olson, 1987 (34 CFU), Rosenzweig, Minnigh and Pipes, 1986 (11 CFU) and West, 1986 (1.5 CFU) for chlorinated water. There are no published reports disclosing data on the types of unchlorinated water (cisterns and ground waters) utilized in this investigation.

As expected of the ground water and cistern samples surveyed, drilled well samples exhibited the fewest number of fungal CFU's (3.2). Recovery values were highest in cisterns (291.0 CFU), with dug wells and springs demonstrating only 78.1 and 56.0 CFU's respectively. The possible reason for high CFU recovery rates in cisterns could be the water contact with environmental surfaces harboring fungi.

Source water for the university (335 CFU) and MUPB

(43 CFU) treatment systems maintained high recovery values for fungi during the test period. This study does indicate that both MUPB and MSU successfully reduced the number of fungal propagules during the treatment of the source water. The municipal treatment process using chemical coagulation and disinfection was more efficient (99.7%) than the university system utilizing rapid sand filatration (96.5% reduction). These findings are consistent with Niemi, Knuth, and Lundstrom (1982) who found that sand filtration allowed more fungal propagules to escape the treatment process than chemical coagulation. However, distribution water in the university system demonstrated a 1.3 fold increase, the municipal system exhibited a 5.0 fold increase. Excluding those samples collected from the MUPB shop, which had a recovery value of 6.75 CFU, a 3.0 fold increase in filamentous fungi was observed in the distribution system. The MUPB shop is located at the end of the distribution line, pipes are made of cast iron, and chlorine levels are low (.38 ppm). Chlorine levels for the MUPB clearwell averaged 0.80 ppm; the level dropped to 0.60 ppm in the distribution system. The university clearwell samples maintained an average of 1.15 ppm of chlorine, but levels fell to 0.7 ppm in distribution lines. The increase within the distribution systems could be due to the lack of contact

time with high chlorine (1-3 ppm). Providing there is enough time, doses in the range of 1 to 2 ppm free chlorine are probably adequate for inactivation of fungal propagules suspended in water. However, 1 to 2 ppm of free chlorine is an unusually high concentration for most water distribution systems and practically all distribution systems have sediment, joints, and/or corrosion tubercles which provide protective habitats from chlorine in the water (Rosenzweig, 1986).

The hemodialysis deionization and reverse osmosis purification unit effectively reduced chlorine levels by 56%. However, the reduced chlorine in the holding tank (0.35 ppm) may permit fungi and bacteria which survived the purification process to recover and reproduce if held in the tank for 24 hours. Two of the four samples collected prior to treatment were positive and demonstrated Fusarium growth (1.0 CFU). The average chlorine for pre-purified water was 0.80 ppm. All samples from the dialysis clinic holding tank were positive for filamentous fungi (35.5), yeast (283.3) and bacteria (432,700). Filamentous fungi most prevalent were Acremonium (49.6%) and Exophiala (37.1%); Rhodotorula (yeast) was isolated as well as Pseudomonas. Rhodotorula fungemia is most often found colonized in catheters, contaminated intravenous solutions, blood tank apparati, and heart-

lung dialysis machines. The affected patients may present endotoxic shock and blood cultures may be positive (Rippon, 1982). Pseudomonas, the bacteria recovered, is a pyrogen and is second only to E. coli as the major cause of hospital associated diseases (Boyd and Hoerl, 1986).

The majority of fungal genera isolated and identified from both the chlorinated and unchlorinated systems belonged to the class Deuteromycetes. These genera are widely distributed and occur in a variety of aquatic habitats, such as lake water (Meyers et al., 1970) and marine zones (Bergen and Wagner-Mernen, 1977).

The most frequently isolated and most numerous genera (Acremonium, Cladosporium, Fusarium, Exophiala, Phoma) observed in this study were in general agreement with Bays, Burman and Lewis (1970), Nagy and Olson (1982), Rosenzweig, Minnigh and Pipes (1986) and West (1986). Differences in genera (Pestalotia and Sporothrix) recovered could be due to the influence in the isolation media and/or climatic and water quality differences between the study locations.

Excluding the dialysis clinic's purified water (35.5 CFU's), samples from the other health facilities demonstrated little fungal growth. The mean CFU's for the hospital and life care center were 0.66 and 0.5, respectively. However, Acremonium, Penicillium,

Cladosporium and Aspergillus isolates recovered from the health facilities have been reported to cause disease in immunosuppressed patients (Rippon, 1982; Emmons, 1977; Beneke and Rodgers, 1980).

Of the 27 genera identified, 22 have been shown to cause disease (Beneke, 1980; Rippon, 1982). Acremonium (Cephalosporium) has been recovered from many cases of mycetoma, onychomycosis, mycotic keratitis, and in rare cases of meningitis (Rippon, 1982). Fusarium spp. are common soil organisms and plant pathogens. In man, Fusarium is most often associated with the colonization of burnt skin and may cause septicemia (Beneke, 1980). Fusarium should be recognized as a potential cause of deep fungal infections in immunocompromised patients. Fusarium infections have been linked with acute leukemia and is considered a new fungal infection in cancer patients (Mycology Observer, 1985). Fusarium, Cladosporium and Aspergillus will readily penetrate contact lenses (Mycology Observer, 1987).

A comparison of isolation media revealed that RBA demonstrated higher recovery values and recovered more genera than CZA during the 7 day incubation period. This may be contributed to RBA's higher nutrient contents.

Total data analysis, using single and multivariate regression, found fungal populations to be significantly

correlated with HPC and chlorine levels. However, no correlation was found between fungi present and total coliforms recovered. Therefore, HPC, as well as fungi populations, may be more suitable microbiological indicators of water quality.

Because fungi occur in a variety of aquatic environments, it is not surprising that they can be readily recovered from drinking water distribution systems. They may enter these systems by a variety of avenues, such as 1) passing through the treatment plant, 2) aerial contamination of service reservoirs or 3) soil contamination of pipelines. Once in the distribution system, fungi may establish themselves on pipe surfaces or in sediment which can collect in the bottom of pipelines. Although fungi can be isolated from distribution systems and unchlorinated water, the role they may play in relation to human health merits further investigation.

CHAPTER VI

SUMMARY

This study showed that fungi are important aquatic organisms. The major results of this study are outlined below:

1. Opportunistic fungi are present in chlorinated and unchlorinated waters and can be isolated from various distribution points including health facilities.
2. Although both treatment plants were effective in fungal removal, rapid sand filtration allowed more fungal propagules to escape into finished water.
3. Of unchlorinated samples, drilled well water recovered fewer fungi (CFU's) than cisterns, springs, and dug wells.
4. The deionization and reverse osmosis purification unit effectively reduced chlorine from the water and allowed for the increase of fungi, yeast and bacteria.
5. Chlorine levels and HPC are significantly correlated to fungi titer.
6. Twenty-two of 27 genera isolated have been shown to cause disease.
7. RBA had better fungal recovery values than CZApek-dox agar in the seven day incubation period.

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